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Operating Instructions

BioPAT® Trace

2-Channel Online Analysis System

85032-541-53

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Important Note:
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Explanation of Symbols

Notes with useful information and user tips.

Warning of potential consequential damage.

Warning of dangerous electrical voltage.

Warning of materials that are health hazards or irritants.

1. Introduction

BioPAT® Trace is a 2-channel online analysis system used to determine glucose and L-lactate. Its applications cover both microbial fermentation processes as well as the cultivation of animal or human cell lines.

When analyzing substrates in cultivation media, it is necessary for a sample to be removed from the bioreactor (fermenter) under sterile conditions. The reproducibility and relevance of the sample taken must remain intact during this step. The BioPAT® Trace currently features the following sampling options: Filtration probe and dialysis probe. All systems enable sterile removal from the bioreactor.

The BioPAT® Trace consists of a compact housing that is adapted to the application and ambient conditions. All mechanical and fluid elements are arranged on the front and | or on the side panels. The electronics feature a modular design and are configured to communicate through several interfaces.

Analysis is carried out using a sensor unit consisting of an electrochemical biosensor. System control and data analysis are run on an external computer. The control software included is very easy-to-use.

2. BioPAT® Trace Product Description

The BioPAT® Trace analysis system is used for simultaneous online monitoring of the analytes glucose and lactate in laboratory or industrial cultivations of microorganisms and cell lines.

An additional application for the measurement of ethanol or methanol is available.

The BioPAT® Trace provides continual analysis independent of the type of cultivation (batch, fed-batch, continual cultivation).

The measurement range of the BioPAT® Trace extends from 0.1 to 40 g/L for glucose, from 0.05 to 10 g/L for lactate, from 0.5 to 20 g/L for methanol and from 1 to 40 g/L for ethanol.
Depending on the conditions, the measurement frequency is 60 analyses per hour maximum. Due to its structured design and easy-to-operate concept, the user can perform routine analysis functions after just a brief introduction.

The ambient temperature of the BioPAT® Trace can range between 15 and 35°C due to internal temperature correction. The ambient humidity should not exceed 90%.

Analysis in the BioPAT® Trace is a combination of enzymatic conversion and electrochemical detection. A 2-channel enzyme electrode coated with oxidase or a combination of an enzyme reactor with a platinum electrode (methanol | ethanol application) serves as the biosensor.

Depending on the application, the service life of the sensor system ensures 30 days or 5,000 measurements. The deviation from the average measurement is less than 3% for a measurement of 5 g/L glucose and 2.5 g/L lactate.

2.1 The Basics of Electrochemical Measuring Technology

An electrochemical detector measures the current produced at a specific potential applied to the electrodes by the analytes in a flow measurement cell. In the presence of an electrolyte solution, the electrochemical conversion of the substance at the working electrode leads to the release or attraction of electrons (oxidation or reduction). The size of the measurable threshold current depends on the parameters of Faraday's law of electrolysis as well as on Fick's diffusion law and is proportional to the concentration.

The selectivity of the sensitive detector is based on the principle that not all substances are electrochemically active and thus many extraneous substances in a sample solution will not be detected. Selectivity is dependent on the working electrode's material and the applied potential.

By combining this system with enzymes, the selectivity of an electrochemical sensor can be raised even higher – and the electrode is turned into a biosensor.

2.2 Biosensory Principle

The analytes glucose and lactate are detected by means of enzymatic reactions. Figure 1 shows the enzymatic conversion of glucose to gluconolactone. In the presence of water, gluconolactone is immediately hydrolyzed to gluconic acid. The hydrogen peroxide (H_2O_2) produced in the first step is detected amperometrically through anodic oxidation that releases the two electrons. Figure 2 shows the enzymatic conversion of lactate to pyruvate. During this reaction as well, H_2O_2 is formed and then detected amperometrically.

Enzyme reactions take place according to the "key-lock principle". They are specific and thus highly selective. Therefore, the reaction at the enzyme system is a further selection. The combination of the two selective principles makes the BioPAT® Trace largely insensitive to matrix effects and extraneous materials.

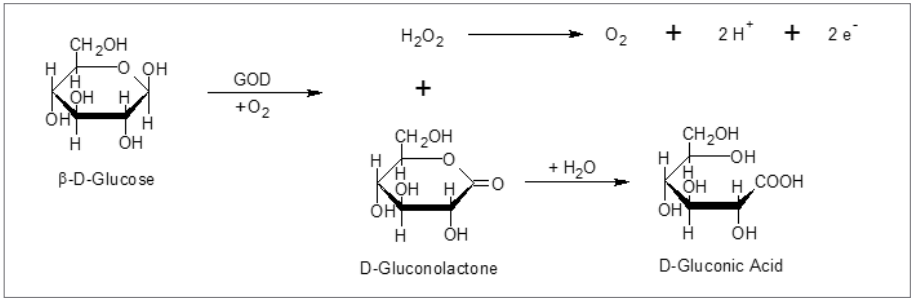

Figure 1: Enzymatic conversion of glucose with parallel anodic oxidation of H_2O_2

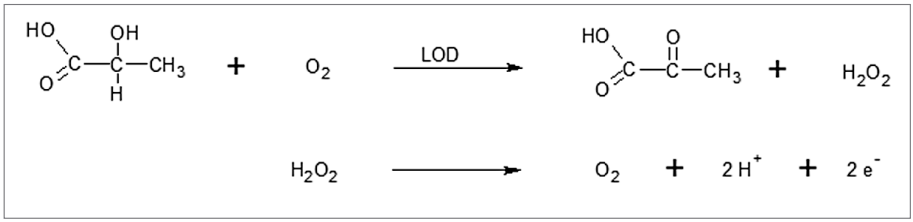

Figure 2: Enzymatic conversion of lactate with parallel anodic oxidation of H_2O_2

The BioPAT® Trace biosensors consist of platinum thick-film electrodes that are durably coated with the corresponding enzymes (glucose oxidase (GOD) or lactate oxidase (LOD)). Then, enzyme and electrode are in close proximity to each other, so that the reactions described above can be recorded directly.

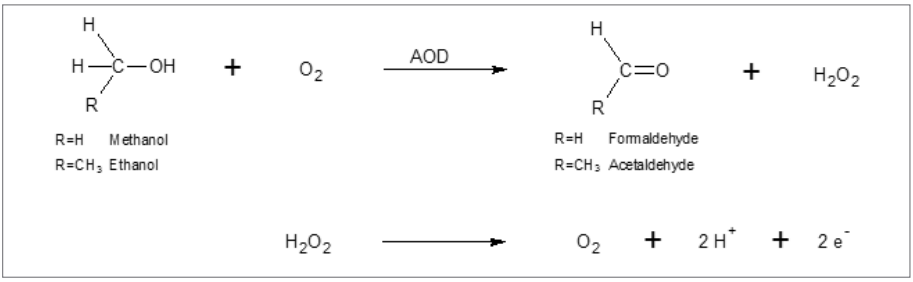

Figure 3: Enzymatic conversion of methanol|ethanol with parallel anodic oxidation of H_2O_2

Figure 3 shows the enzymatic conversion of methanol or ethanol to formaldehyde or acetaldehyde. The enzyme alcohol oxidase (AOD), which occurs in an enzyme reactor in immobilized form, is used as catalyst. The hydrogen peroxide (H_2O_2) formed in the reaction is detected amperometrically. With this method, the alcohol is converted in the enzyme reactor and the detection of the formed hydrogen peroxide takes place on a platinum electrode.

2.3 Fluidic System and Sampling

To perform the respective analysis on a sample, representative material must be transported to the biosensor. A fluidic system (tubing set) consisting of tubing lines, pumps and valves is used for that purpose. Additionally, the sample is also conditioned in the fluidic system, i.e. potentially interfering components are removed, and diluted to the extent that it can be assayed reliably by highly sensitive biosensors. This is done by separation through a dialysis membrane, which is integrated in every tubing set as a diffusion module.

Conditioning of the sample is based on the method of flow diffusion analysis (Figure 4). This method works with a time-dependent sample transfer. For that purpose, the measurement line and the sample and | or the sample infeed are separated from each other by a membrane. The measuring distance is called the acceptor (receiving) and the sample side is called the donor (giving).

During an analysis, the measurement line is flushed with a transport buffer, which is an acceptor stream that can be stopped from behind the membrane for a defined period of time. This procedure is carried out by triggering a valve, which in turn opens up a bypass route.

Meanwhile, the analytes in the sample diffuse through the membrane and, at the same time, are enriched in the holding chamber. In this way, a continually increasing concentration of dialysable components develops in the fluid segment of the transport buffer.

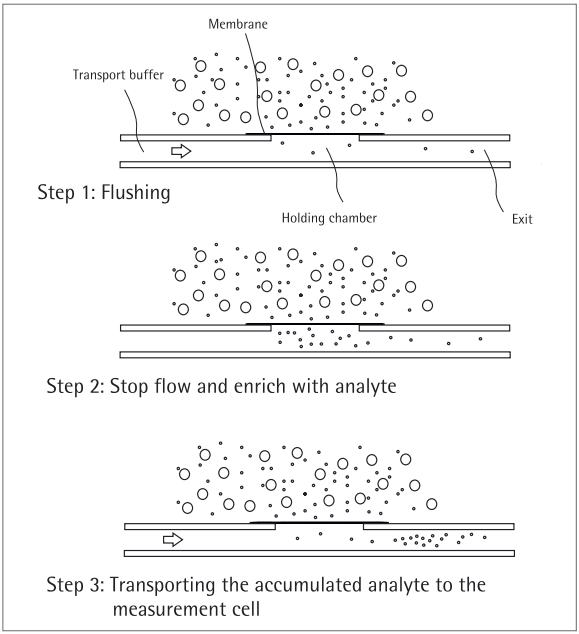

Figure 4: Principle of the flow diffusion technique

When this segment is released by re-triggering the valve, the flow goes to the measurement cell where it generates a signal. The intensity of the signal is a measure of the concentration of the sample. One big advantage of this enrichment method is the very broad measuring range that is achieved by varying the diffusion time; it also protects the sensor against inferring particles in the sample.

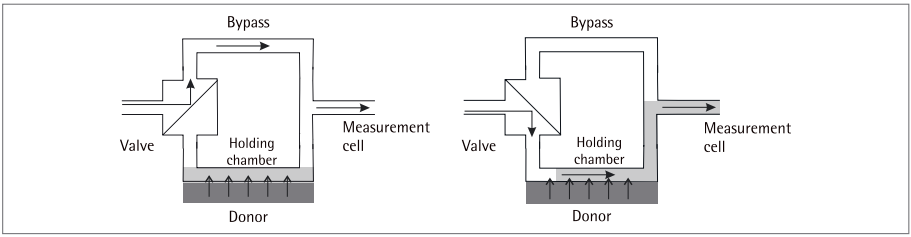

Figure 5: Flow diagram to illustrate flow diffusion analysis

Image on the left: Valve control during the determination of the baseline and while loading.
Image on the right: Valve control while unloading and during signal detection.

2.4 Data Transmission and Data Saving

The BioPAT® Trace has a number of outputs, making integration into data recording systems very flexible. Along with a standard analog output for signal ranges from 0 to 20mA, 0 to 10 V or 4 to 20mA, the BioPAT® Trace also features an Ethernet connection that connects the device to a PC via a network. Using the software supplied, the measured results can be visualized graphically and saved (see Chapter ▶"7. Operation" on page 85).

3. General Safety Precautions

The BioPAT® Trace was designed and built under consideration of hazard analyses, additional technical specifications and according to a meticulous selection of harmonized standards to be complied with. It is thus a state-of-the-art device that ensures the highest degree of safety.

However, this safety can only be achieved during practical operation when all the necessary measures are applied. It is the operator's responsibility to plan these measures and monitor their execution.

In particular, the operator must ensure that
- the BioPAT® Trace is used for it's intended purpose only (cf. Chapter ▶"2. BioPAT® Trace Product Description" on page 12),
- the BioPAT® Trace is used only when functional and in proper working order,
- the operating instructions are always kept legible and complete at the place of use,
- the BioPAT® Trace is serviced and repaired by adequately qualified personnel only.

The symbols used in the operating instructions are specifically intended to draw attention to the safety precautions! The symbol may not replace the text of the respective safety precaution.
Therefore, the text must always be read completely!

Indicates useful information and user tips.

Warning of potential consequential damage.

Warning of dangerous electrical voltage.

Warning of materials that are health hazards or irritants.

4. Equipment Supplied, Accessories and Consumables

4.1 Equipment Supplied

Description	Order No.
Online Analytical System BioPAT® Trace with power adapter, power plugs (Europe, USA, UK), cross-over ethernet cable, operationg instructions and PC software (trace_mon)	BPT0001

Table 1: Equipment supplied with BioPAT® Trace

4.2 Consumables

4.2.1 Tubesets

Description	Order No.
Tubeset Dialysis (Glucose | Lactate)	BPT0003
Tubeset Filtration (Glucose | Lactate)	BPT0004
Tubeset Filtration (Glucose | Lactate | cellulase resistant)	BPT0005
Tubeset Dialysis (Ethanol | Methanol)	BPT0050
Tubeset Filtration (Ethanol | Methanol)	BPT0049
Enzyme reactor for Application Ethanol | Methanol	BPT0048

4.2.2 Transport Buffer Concentrate

Description	Order No.
Transport buffer concentrated 20× (for cell cultivations), Application Glucose | Lactate, 0.5 liter	BPT0006
Transport buffer concentrated 5× (for microbial cultivations), Application Glucose | Lactate, 1 liter	BPT0060
Transport buffer concentrated 5×, Application Ethanol | Methanol, 1 liter	BPT0046

4.2.3 Disinfectant Concentrate

Description	Order No.
Disinfectant concentrate, 1 liter	BPT0044

4.2.4 Calibration Standard (content: 0.5 liter)

Description	Order No.
Calibration standard 0.1 g/l Glucose/ 0.05 g/l Lactate	BPT0043
Calibration standard 0.5 g/l Glucose/ 0.25 g/l Lactate	BPT0011
Calibration standard 1 g/l Glucose/ 0.5 g/l Lactate	BPT0010
Calibration standard 2 g/l Glucose/ 1 g/l Lactate	BPT0009
Calibration standard 4 g/l Glucose/ 2 g/l Lactate	BPT0008
Calibration standard 10 g/l Glucose/ 5 g/l Lactate	BPT0007
Calibration standard 20 g/l Glucose/ 10 g/l Lactate	BPT0041
Calibration standard 0.2 g/l Methanol	BPT0051
Calibration standard 1 g/l Methanol	BPT0052
Calibration standard 5 g/l Methanol	BPT0053
Calibration standard 20 g/l Methanol	BPT0054
Calibration standard 0.5 g/l Ethanol	BPT0058
Calibration standard 2 g/l Ethanol	BPT0057
Calibration standard 4 g/l Ethanol	BPT0056
Calibration standard 40 g/l Ethanol	BPT0055

4.2.5 Membranes

Description	Order No.
Membranes for Dialysis probe (Glucose | Lactate); 5 pieces	BPT0024
Membranes for Dialysis probe (Glucose | Lactate | cellulase resistant); 5 pieces	BPT0040
Membranes for Dialysis probe (Ethanol | Methanol); 5 pieces	BPT0047
Membrane for Filtration probe, length: 130 mm	BPT0025
Membrane for Filtration probe, length: 90 mm	BPT0037

Table 2: Consumables for BioPAT® Trace

4.3 Sampling Systems

4.3.1 Dialysis Probe (delivery with installation kit, 2 membranes and user manual)

Description	Order No.
Dialysis probe length: 132 mm	BPT0014
Dialysis probe length: 165 mm	BPT0015
Dialysis probe length: 212 mm	BPT0016
Dialysis probe length: 232 mm	BPT0017
Dialysis probe length: 332 mm	BPT0018
Dialysis probe length: 362 mm	BPT0019
Dialysis probe length: 432 mm	BPT0020

4.3.2 Single-Use Dialysis Probe (delivery with user manual)

Description	Order No.
Single-Use Dialysis probe ContiTRACE	BPT0002

4.3.3 Filtration Probe (delivery with installation kit, 1 membrane and user manual)

Description	Order No.
Filtration probe for cover port 12 mm with retaining nut PG13.5, membrane length 130 mm	BPT0021
Filtration probe for cover port 12 mm with retaining nut PG13.5 (insertion depth extended by 100 mm), membrane length 130 mm	BPT0022
Filtration probe for side port 25 mm with shut-off cock, membrane length 130 mm	BPT0023
Filtration probe for side port 25 mm with shut-off cock, membrane length 90 mm	BPT0036

Table 3: Sampling systems for BioPAT® Trace

4.4 Accessories for Sampling Systems

4.4.1 Accessories for the Dialysis Probe

Description	Order No.
Installation kit Dialysis probe	BPT0045
Adapter 25/12 mm for the installation of the 165 mm dialysis probe in 25 mm side port (Ingold port 52 mm) incl. o-rings	BB-38242540

4.4.2 Accesories for the Filtration Probe

Description	Order No.
Installation kit Filtration probe	BPT0059
Tubeset for hydrophilization of filtration membranes	BPT0042
Membrane protective cage for filtration probe with membrane length 130 mm for the installation in 25 mm side port (insertion depth extended by ca. 25 mm)	BPT0038
Membrane protective cage for filtration probe with membrane length 90 mm for the installation in 25 mm side port (insertion depth extended by ca. 25 mm)	BPT0039

4.4.3 Accessories for the Dialysis and Filtration Probe

Description	Order No.
Retaining nut PG13.5 with o-ring and sliding ring	BPT0061
Adapter 19/12 mm for the installation of the dialysis and filtration probe in 12 mm cover port (M26×1); incl. o-rings	BB-8848630
Adapter 19/12 mm for the installation of the dialysis and filtration probe in 12 mm cover port (RD28×1/8")	BB-34104704
O-ring (outside) for adapter RD28×1/8"	BB-39120830
O-ring (inside) for adapter RD28×1/8"	BB-39121054

Table 4: Accessories for sampling systems

4.5 Accessories for BioPAT® Trace

Description	Order No.
Stand	BPT0012
Stand clamp	BPT0013
Knurled screw for measurement cell (replacement)	BPT0026
Power supply	BPT0027
Power cable (EU, UK, USA)	BPT0028

Table 5: Accessories for BioPAT® Trace

5. Design and Function

5.1 Setting Up the Device

The BioPAT® Trace is an online analysis system in a compact metal housing. Communication with the user is driven by an external computer. As shown in Figure 6 and 7, the front and side panels are equipped with the elements of the pre-assembled tubing set for analysis.

The actuator and sensor components are located on the outside of the housing. A standard or sample solution is fed into the analysis system by a pump (1 b). One of the pinch valves (3) allows a selection of the solutions to be pumped.

The buffer stream is driven by the upper pump (1 a). A particle filter (2) removes smaller particles and air bubbles from the buffer stream.

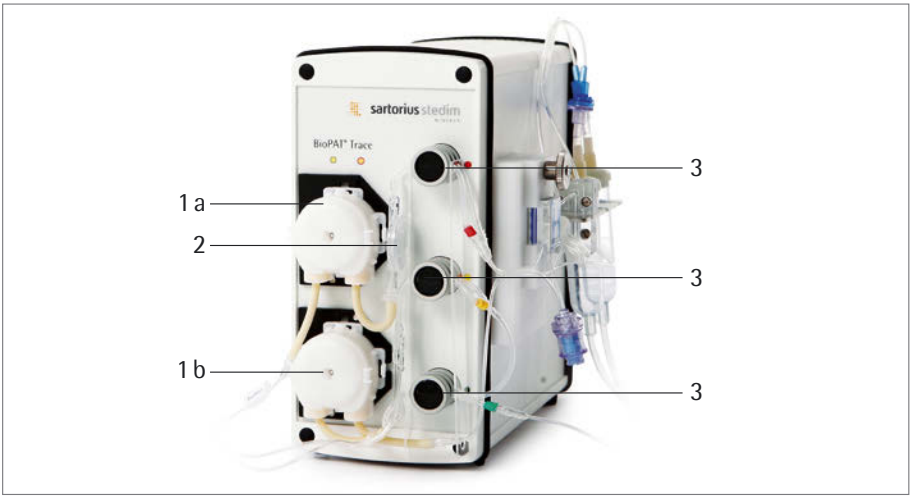

Figure 6: Front view of the BioPAT® Trace with the following components:

Pos.	Component
1 a | b	Pump cassettes (2 units)
2	Particle filter
3	Pinch valves (3 pcs.)

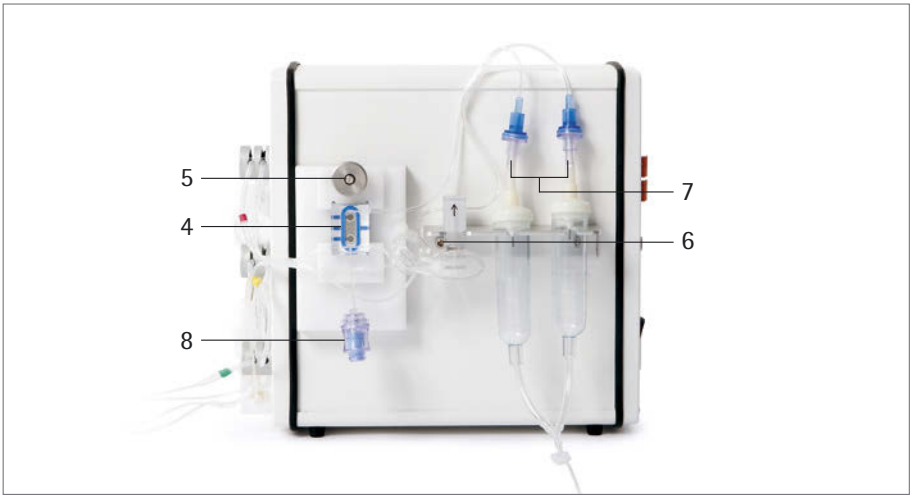

Figure 7: Side view of the BioPAT® Trace with the following components:

Pos.	Component
4	Measurement cell
5	Knurled nut for fastening the measurement cell
6	Diffusion module
7	Drip counters
8	Rinsing line with return valve

In the diffusion module (6), a segment of the buffer solution is enriched with the analyte from the donor stream. After time-dependent enrichment in the diffusion module (6), the acceptor stream flows into the measurement cell (4) for assaying the analyte. The measurement cell is fixated by a retaining plate, which can be fastened or loosened with a knurled nut (5). Furthermore, a rinsing line with return valve (8) is attached to the measurement cell. Before the solutions are transported to waste, they run through a drip count (7).

Figure 8 shows the rear view of the BioPAT® Trace. The device can be switched on and off by pressing the power switch (9).
Using the calibration data, the electrochemical signal of the biosensor is converted internally to a concentration value. The measured value is output after the analysis is completed on a PC interfaced to the Ethernet connection (10). Optionally, the signals can be output via the analog signal output (12). The following ranges are possible: 0..10 V, 0..20 mA, 4..20 mA.
The USB interfaces (11) and the serial interface (RS232 port) (13) can also be used for data transfer (currently not implemented).

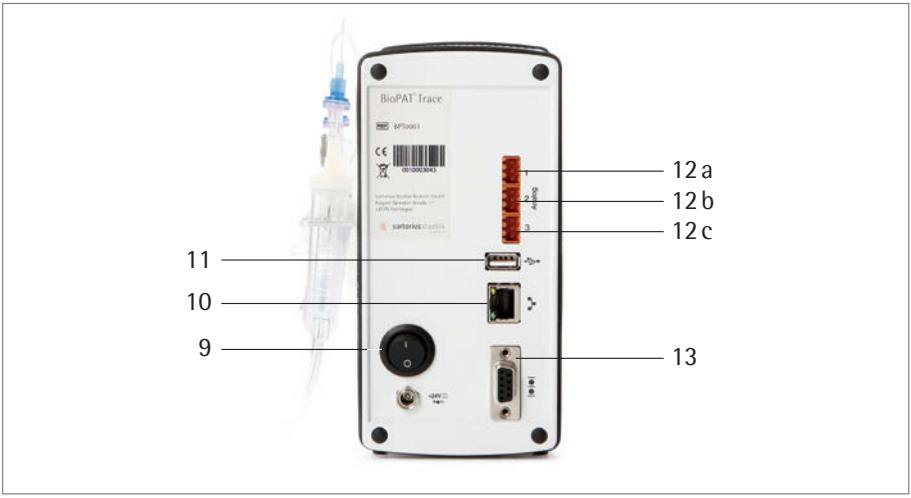

Figure 8: Rear view of the BioPAT® Trace with the following elements:

Pos.	Function
9	Power switch
10	Ethernet connection
11	USB interface
12 a | b | c	Analog outputs (1–3)
13	Serial interface

The BioPAT® Trace can optionally be attached to laboratory stands, suspension bars or benchtop stands. A corresponding stand clamp and a suitable benchtop stand are available as accessories.

Under no circumstances should a voltage source be connected to the analog data outputs at the back of the BioPAT® Trace. This also refers to a multimeter for resistance measurement.

If a multimeter is used to measure the voltage or current, the contacts to the BioPAT® Trace must be removed before changing the measurement range on the multimeter. Otherwise, there is the danger that the device will be damaged.

5.2 BioPAT® Trace Measurement Methods

Two online measurement methods are possible with the BioPAT® Trace:
– Filtration with a filtration probe,
– Dialysis with a dialysis probe.

The simplest method is to directly measure a filtered sample of medium. However, because reactor medium is used, the range of applications is limited to processes for which there's a sufficient reactor volume or which allow continuous-feed.

Dialysis sampling is an option when processes are involved for which reactor volume does not allow enough sample material.
This method only removes low molecular substances from the reactor medium, without reducing the volume of fluid. For example, Figure 9 shows a photographic representation of how the BioPAT® Trace can be connected to a bioreactor by way of a dialysis probe.

A more detailed explanation of the sampling probes can be found in Chapters
▶ "5.6 Dialysis Probe Function" on page 37,
▶ "5.7 Filtration Probe Function" on page 38.

Figure 9: Principle of sample infeed via the dialysis probe on BioPAT® Trace

5.2.1 Dialysis Measuring Method Using the Dialysis Probe

The dialysis measuring method using the dialysis probe involves the transfer of analytes through a dialysis membrane in the dialysis probe which separates the reactor contents (medium) from the acceptor stream. At the same time, the dialysis membrane functions as a sterile barrier.
Figure 10 shows a schematic of the flow system.

The calibration valve (green) and the three-way stopcock can be used to choose between the standards and the manual probe. The sample valve (red) and the bypass valve (yellow) can be used to select the dialysis probe and the diffusion module.

The upper part of the flow system represents the donor stream, which is responsible for the infeed of the calibration solution into the diffusion module. Depending on the task at hand, the carrier stream accumulates the analyte in the diffusion module or in the dialysis probe.

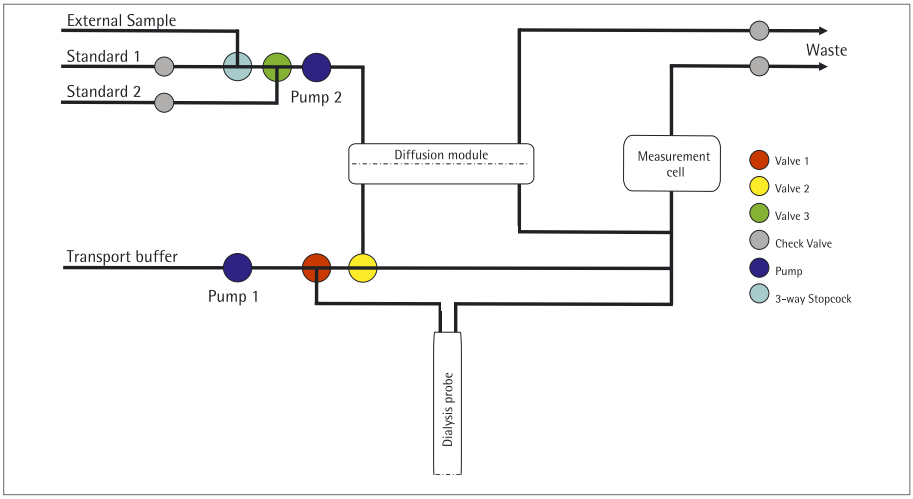

Figure 10: Flow diagram of the online dialysis method

For the dialysis method, the internal diffusion module is used to calibrate the BioPAT® Trace. By contrast, the dialysis probe in the bioreactor is always used for measuring the sample. A reference factor between the membranes is then determined once.

As a standard cannot be installed in the bioreactor, the BioPAT® Trace must be calibrated indirectly by way of the reference factor. During this process, the initial concentration in the bioreactor is used as the starting concentration for the dialysis probe. Using the sample measurement values with an identical concentration, the BioPAT® Trace calculates the factor between the diffusion module membrane and the dialysis probe membrane.

Hence, the calibration procedure for the dialysis method consists of three steps in total:

Step 1:
Recording the calibration curves on the diffusion module with standards 1 and 2

Step 2:
Measuring the external sample on the BioPAT® Trace with the help of the calibration curves (alternatively: by directly entering the concentration values, if known from the initial weight or by external analysis)

Step 3:
Recording the calibration curves of the dialysis probe using the probe

After measuring the standards for calculating the calibration curves, the BioPAT® Trace computes a factor from the gradients of each individual curve. For all measurements carried out with the dialysis probe, this factor is applied to calculate the true concentration.

5.2.2 Filtration Measuring Method Using the Filtration Probe

With the filtration measuring method, the samples are drawn from the bioreactor through the filtration probe. Here, transferral of the sample into the buffer stream takes place by means of diffusion within the diffusion module. The calibration valve (yellow) and the sample valve (green) are used to select between the calibration solutions and the filtration probe. The bypass valve (red) is used to select the diffusion module and the bypass. Figure 11 shows a schematic of the flow system.

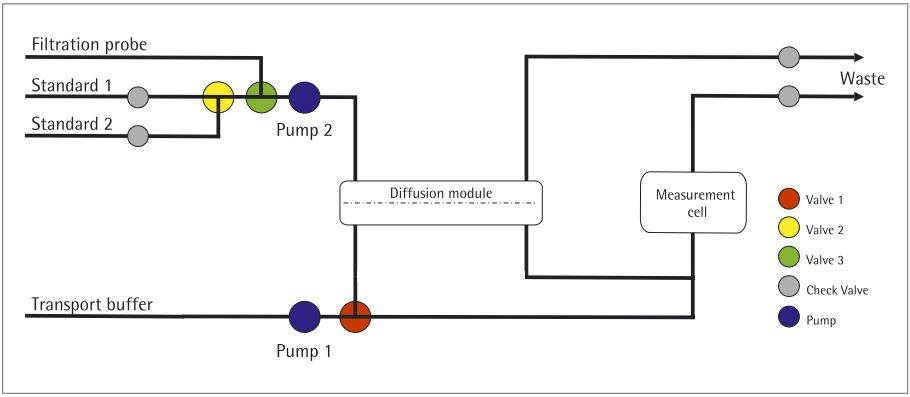

Figure 11: Flow diagram of the online filtration method

With the online filtration method, the diffusion module is always used for calibrating the BioPAT® Trace and for measuring samples. The BioPAT® Trace calculates the regression curve including zero point and gradient from multiple measurements of calibration standards 1 and 2. In turn, the sample concentration is calculated based on the calibration curves.

Figure 12:
Measurement cell with rinsing line

5.3 Measurement Cell Function

The measurement cell contains biosensors for glucose and lactate. The measurement cell is shown in Figure 12. The fluid inlet flow runs perpendicular to the 2-channel sensor ("wall jet" principle).
A viewing window allows the operator to monitor the air bubble-free flow through the cell. Any air bubbles can be removed as necessary by rinsing the measuring chamber. For that purpose, the fluid is injected through the rinsing line equipped with a return valve.

Contact springs intercept the electric signal measurable at the electrodes and transmit it to the BioPAT® Trace. The measurement cell is fastened to the device with a knurled nut.

It must be ensured that no air bubbles are in the measuring chamber of the flow cell, as these can influence the results. After connecting the sampling probe and before starting each measurement series, check the measurement cell for air bubbles through the viewing window. Any existing air bubbles must be removed with the help of the rinsing line.

Use only transport buffers or distilled water to rinse the measurement cell! Other fluids can damage the sensor. Use only fresh solutions and | or a sterile filter attachment in order to prevent microbes from entering the tubing set!

5.4 Particle Filter Function

The particle filter is part of the tubing set. It fulfills two tasks in the BioPAT® Trace. First, sterile filtration of the buffer solution is performed through the membrane with a pore size of 0.22 µm. Before the solution leaves the module, a second membrane is used for degassing. Thus, the particle filter ensures that the acceptor stream in the BioPAT® Trace stays free of particles and air bubbles.

To ensure proper operation of the measuring instrument, the tubing set, including the particle filter, must be replaced regularly.

5.5 Diffusion Module Function

The diffusion module is part of the tubing set. The standard solutions or an external sample are pumped through the diffusion module to the analysis system. A membrane enables diffusion of the substrate from the donor solutions (both standard and sample) into the buffer solution (acceptor). The different membrane materials enable dialysis, cellulase-stabile dialysis and gas diffusion for separating substrates. Figure 13 shows a schematic diagram of this system.

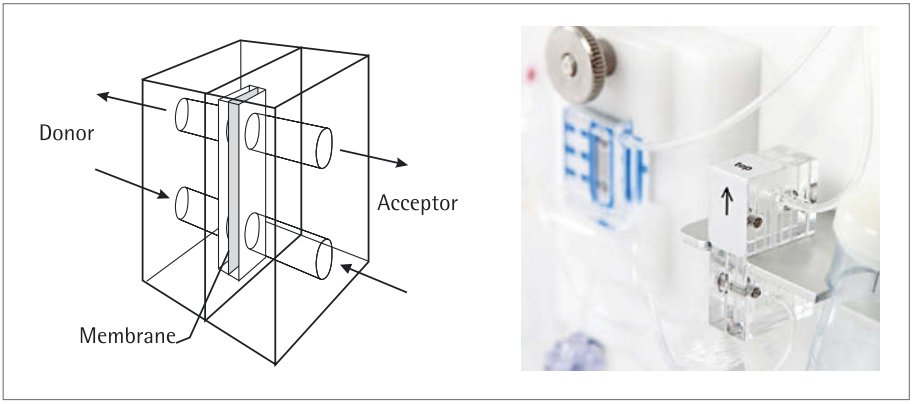

Figure 13: Schematic design and fixation of the diffusion module

The module is made of PMMA. It has a diffusion area of 20 mm^2 and ensures that a sufficient exchange of substances takes place between the donor and the acceptor stream. The donor and acceptor streams flow in through the lower module inlet. The drain is located at the top.

In order to ensure reliable operation of the measuring instrument, the diffusion module must be secured in the correct position on the retaining plate (arrow pointing up).

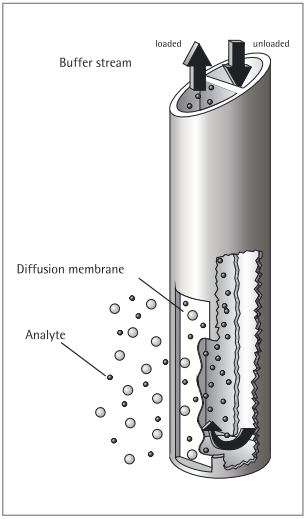

Figure 14: Functional principle of the dialysis probe

5.6 Dialysis Probe Function

If a dialysis probe is used to feed the sample from the bioreactor into the BioPAT® Trace, then the analyte is transferred through a diffusion membrane into an internal pump-driven buffer stream and transported to the measurement cell. Figure 14 shows how the principal function of how the probe works.

The dialysis probe is suitable for all types of cultivation. The use of an extremely robust diffusion membrane ensures a high level of sterility. The different membrane materials enable dialysis, cellulase-stabile dialysis and gas diffusion for separating substrates. The probe can be installed in the bioreactor as well as in the cover or in a side nozzle as well.

The application options for the dialysis probe depend on the reactor dimensions and the process conditions. In general, the dialysis probe is recommended for use on smaller bioreactors, where any loss of volume during sampling is unacceptable. The use of the dialysis probe as a sampling system ensures analysis at constant volumes.

5.7 Filtration Probe Function

Figure 15: Filtration probe for sterile sampling from bioreactors

Cell-free substrate samples are removed from the bioreactor using the sampling probe on a filtration basis (Figure 15). The hydrophilized probe is mounted inside the bioreactor and subsequently sterilized along with the medium. The membrane module of the probe is positioned in the turbulent zone of the reactor, near the agitator. A tubular membrane made of polypropylene serves as a sterile barrier. The filtrate flux does not exceed 2 mL/min.
After sampling, the cell-free sample can be fed directly into the BioPAT® Trace for analysis. The filtration probe is used when a sufficient reactor volume is available.

Figure 16: AOD enzyme reactor

5.8 Enzyme Reactor Function

The enzyme reactor is required for measuring methanol and ethanol (Figure 16). Inside the reactor is the immobilized enzyme alcohol oxidase (AOD). There, the corresponding alcohol is converted and hydrogen peroxide is formed. The hydrogen peroxide is then detected in the measurement cell.

Due to the stability of the enzyme used, the ambient temperature may not exceed 25°C. At higher temperatures, it is recommended to use a climatic chamber.

5.9 Temperature Correction

The BioPAT® Trace is equipped with a temperature sensor. This is arranged in the proximity of the measurement cell. All measured values are corrected by using a temperature-dependent function.

6. Getting Started

6.1 Transport

The BioPAT® Trace including accessories is delivered in protective packaging. Please save this packaging; proper shipping is only possible in the original packaging.

Upon receipt, check the delivery for completeness and any possible damage that may have occurred in transit. Any transport damage should be reported within a week of delivery. Complaints made after this date will not be accepted.

6.2 Setup

6.2.1 Setting up the Equipment

The BioPAT® Trace must be setup near the bioreactor you wish to monitor (at max. distance of 2 m). The location must be free of vibrations and protected against corrosive atmospheres and chemical contamination.
Additionally, the BioPAT® Trace should be protected against exposure to direct sunlight.

Follow the instructions and requirements listed below concerning the power connection. Operating the equipment with the wrong supply voltage and | or the wrong fuses can be life-threatening.

The AC adapter supplied with the device is equipped with a power plug, a mains adapter and a plug to the device. Insert the device plug into the connection socket on the back panel of the BioPAT® Trace and connect the other end to your mains outlet. The device can be switched on and off by pressing the power switch. When the device is switched on, a green LED lights up on the front panel.

To connect the BioPAT® Trace to the local network, plug the Ethernet cable into the Ethernet connection on the back panel of the device. The operating software supplied is used to control the device via a PC. Likewise, this PC must also be connected to the local network or directly to the device using the cross-over Ethernet cable supplied.

After connecting the device to the PC, check to make sure that the system times of the two units are synchronized. Proceed as described in Chapter ▶ "7.1.4.2 Basic Settings" on page 106.

6.2.2 Tubing on the Device

Depending on the type of operation intended, the BioPAT® Trace is equipped with corresponding tubing set (fluidics).

For each of these types of operation, you will find below the corresponding instructions for attaching the tubing set.

The corresponding tubing set consists of the components necessary for analysis: Pump cassettes, valve tubes, measuring chamber, diffusion module, drip counters and connection lines for buffers, standards, waste as well as the sampling probe (filtration, dialysis).

The lines for buffers, standards, sampling probe and waste are premounted and packaged in foil bags. It is recommended to only open these after the tubing set has been attached to the device in order to facilitate handling.

Figure 17: View of a tubing set for the "Filtration" operating mode

In addition, the dialysis tubing set is provided with a single-use syringe with three-way stopcock for the analysis of external samples.

Before you start attaching, remove the two empty cassettes on the pump slots. The empty cassettes can be reused as transport protection for the motor axle.

Only use tubing from the prefabricated original tubing set. To ensure that all solutions flow freely, never allow the tubing segments to be crushed or kinked.

Make sure that the valve tubes are firmly seated in their pinch valves in order to ensure that the tubing can be opened and closed without problems.

The inlet and outlet lines of the tubing set are packaged in foil bags and labeled.
The individual tubes are affixed with the following labels:

– BUFFER	Connect to the transport buffer
– WASTE	Connect to waste
– ←	Probe port 1 dialysis
– →	Probe port 2 dialysis
– Sample	Probe port filtration
– Standard1	Cover label for standard calibration solution 1
– Standard2	Cover label for standard calibration solution 2

Before filling tubing set, make sure that tubing is connected to all solutions and that the vessels are sufficiently filled. Tubing sets that are only partially filled or not filled at all can produce erroneous measurements!

The transport solution is delivered as concentrate. Observe the note for proper dilution on the packaging. Incorrect or no dilution will lead to erroneous measuring values!

Figure 18: View of fully-equipped front panel for the "Filtration" operating mode

6.2.3 Attaching the Filtration Tubing Set

Before attaching the filtration tubing set, the valve tubes must first be inserted into the pinch valves. To keep them from getting mixed up, each line is color-coded. The following rules apply:

- The pair of tubes are color-coded (red, yellow and green) and are kept in place by two guide plates
- The top-to-bottom order follows the traffic light principle: red at the top, yellow in the middle, green at the bottom
- The color-coded line of the pair of tubes is always inserted into the front valve slot
- When inserting the tubing, always guide the labeled side with your right hand

After you have inserted the tubing set, check that the result looks like in this picture.

Figure 19: Tightening and aligning of the pair of tubes in the guide plate

As the first step, take both guide plates in both hands and open them slightly in order to align the valve tubes in parallel (see Figure 19).

The red pair of tubes should be on top and the label on the right should be visible.

Figure 20: Position of the valve tubes on the device

Now place the guide plates on the valves as such that the pair of tubes are positioned on their corresponding valve slots (see Figure 20).

When inserting the tubing, make sure that the red, yellow and green color coding is always located to the right of the valve and matches that valve's color coding.

During insertion, hold both sides of the tubing tautly and press the pair down until both tubes are securely seated in their slots (see Figure 21, 22).
Whilst doing so, press alternately on the black buttons on the valve, to ensure you also insert the rear hoses deep into the valve slot. Then check that the tubes are fitted correctly in the valve in order to avoid measuring errors.

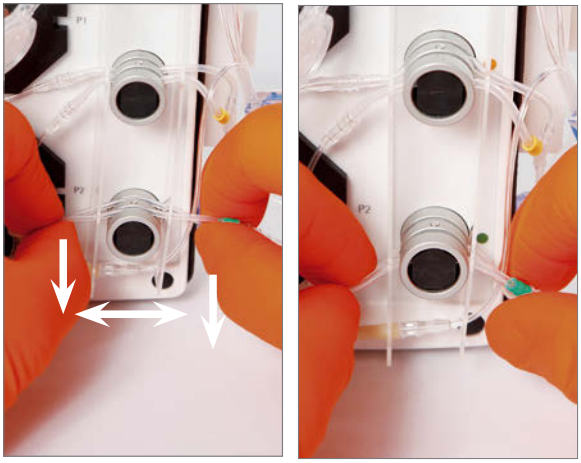

Figure 21, 22: Tightening and inserting the pair of tubes

Figure 23: Securing the valve tubes by pushing together the guide plates

After you are done fitting all three valves, push both guide plates towards the valve in order to secure the position of the tubes in the valve slot (Figure 23).

As the next step, install the two pump cassettes on the motor shaft and press on the retaining plate firmly until you hear an audible click indicating that it has locked in.

Before installing the pump cassettes, check if the motor shaft is clean (if necessary, wipe with a soft cloth). Dirt particles or abrasion of previous applications can adversely affect or even completely prevent the proper functioning of the pump.

The lower pump (P2) and the upper pump (P1) are attached one after the other (see Figures 24, 25).

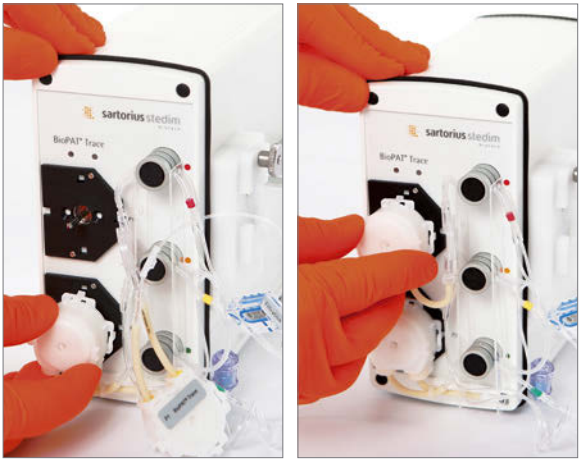

Figure 24, 25: Attaching lower pump cassette P2 and upper pump cassette P1

Figure 26: Proper placement of the pump cassette (fastening stud locked in place)

The proper placement of the pump cassettes should be verified (Figure 26).

Figure 27: Fully assembled side panel

After installing the pumps and valves on the front of the device, attach the sensor, drip chamber and diffusion module to the side panel.

Figure 27 shows a completely assembled side panel.

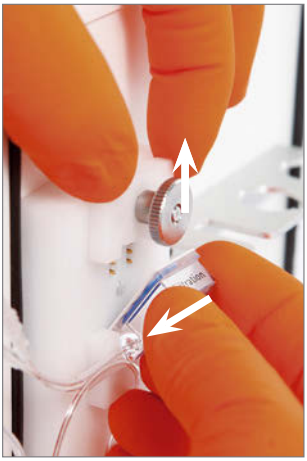

Figure 28: Inserting the measurement cell into the device

First, insert the measurement cell into the measurement amplifier holder (Figure 28).

To this end, unscrew the knurled nut on the upper retaining plate to allow it to be pushed upwards.

Next, place the lower edge of the measurement cell in the recess in the lower border of the retaining plate (Figure 28).

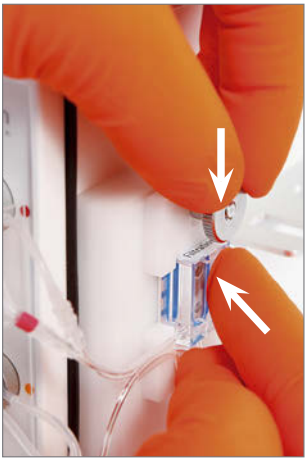

Figure 29: Fastening the measurement cell to the device

Leverage the measurement cell up onto the contact plug. While doing so, pull the upper retaining plate slightly upwards.

Press the measurement cell onto the holder and slide the retaining plate downwards (Figure 29).

The measurement cell is fastened to the device by tightening the knurled nut.

Figure 30: Fastening the diffusion module to the mounting rail

Afterwards, attach the rest of the components to the device. Using the holding slits on the sides, fasten the diffusion module to the mounting rail (Figure 30). Make sure that the diffusion module is properly aligned with the arrow pointing upwards.

Figure 31: Inserting the drip counters into the holder

Next, insert the two drip counters into the holder designed for this purpose (Figure 31).

6.2.4 Attaching the Tubing Set for the "Dialysis" Operating Mode

To install the dialysis tubing set, follow the same procedure as for the filtration tubing set.

Additional information:
In addition, the dialysis tubing set is provided with a single-use syringe with three-way stopcock for the analysis of external samples. If you want to use this option, you have to place the three-way stopcock between the connection with the green label and the line to calibration standard 1 (see Figure 32 and 33).

Figure 32: Place of installation for three-way stopcock

Figure 33: Three-way stopcock and syringe mounted

6.2.5 Connection and Removal of the Enzyme Reactor

In the "Methanol | Ethanol" operating mode, an enzyme reactor with immobilized alcohol oxidase is required for the enzymatic conversion of analytes. Since the enzyme is relatively heat sensitive and cannot be stored dry, the reactor is delivered separately from the tubing set. The enzyme reactor must be installed into the tubing set intended for this purpose. Due to the storage conditions at 4-8°C, this should only be carried out directly prior to initial operation.

Prior to installing the reactor, the tubing set must be inserted into the BioPAT® Trace and filled with buffer. Dry setup may result in decrease of the reactor capacity and should therefore be absolutely avoided. First, start the filling process to fill the tubing set (see Chapter 7, Figure 64). The progress of the filling in the display can be tracked by activating the status display (see Chapter 7.1.3). The device must be stopped prior to or during the preliminary measurements to allow the reactor to be installed. Stop the device with "Stop" (see Chapter 7.1.2.3).

If air enters into the enzyme reactor, there is the danger of a decrease in the capacity of the enzyme reactor in association with a lower sensitivity.

In order to install the enzyme reactor, remove it from the transport container and open the red cover caps. Afterwards, install it into the intended location in the tubing set (see Figures 34-36).

After installing the enzyme reactor, restart the device with "Setting-up Operation" (see Chapter 7.1.2.2). Select "New tubing set" and start another filling process with the installed enzyme reactor.

Figure 34: Place of installation for the enzyme reactor

Figure 35: Securing the enzyme reactor

Figure 36: Fully installed enzyme reactor

The removal of the reactor is also carried out in "stopped" status ("Device – Stop"). The reactor is closed with the red cover caps, placed into the transport container and stored at a cool temperature between 4 – 8 °C.

For further usage, the enzyme reactor must be removed from the tubing set, filled-up with transport buffer and stored in the refrigerator at 4 – 8 °C.
If air enters into the enzyme reactor, there is the danger of a decrease in the capacity of the enzyme reactor resulting in lower sensitivity.

6.2.6 Connecting the Dialysis Probe

6.2.6.1 Mounting the Dialysis Membrane in the Dialysis Probe

Install a new dialysis membrane before every online measurement involving bioprocesses with sterile feeds as shown in the following Figure 37. If this rule is not observed, a lack of sterility may jeopardize the entire cultivation process.

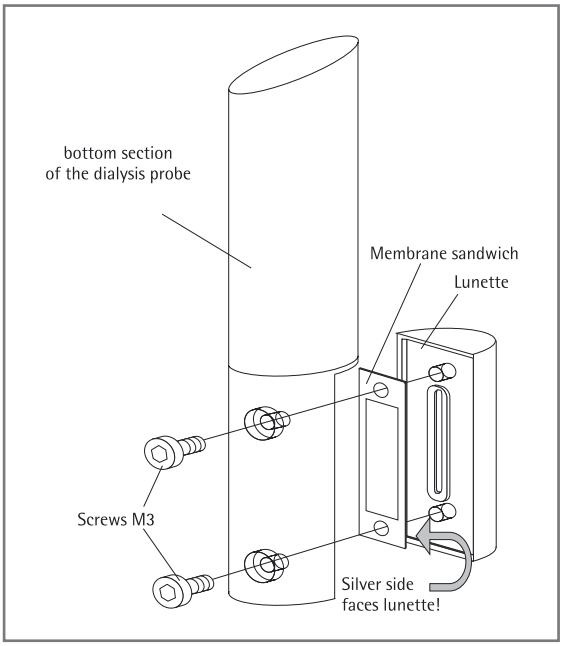

Figure 37: Replacing the membrane in the dialysis probe for the glucose | lactate application

The new dialysis membrane must be placed correctly and the probe lunette must be tightened firmly with the two screws, see Figures 38 to 40.

Figure 38: Inserting the membrane

Figure 39: Installation of the probe lunette

Figure 40: Tightening of 2 screws

Pay particular attention to ensure that the membrane is seated correctly in the dialysis probe.

The white side of the membrane must be inserted facing the dialysis probe (buffer-touching). The silver side of the membrane faces the probe lunette (media-touching).

Incorrect insertion of the membrane may result in leaks in the dialysis probe head and jeopardize the sterility of the entire cultivation process.

6.2.6.2 Installing the Dialysis Probe into the Bioreactor

Insert the friction washer and the O-ring prior to installation in the fermenter (Figure 41).

Figure 41: Inserting the friction washer and O-ring

6.2.6.3 Sterilization of the Dialysis Probe Prior to Connecting to the BioPAT® Trace

Before connecting the dialysis probe to the BioPAT® Trace, the probe must be sterilized along with the bioreactor. Connect the dialysis probe to the tubing set as shown in Figure 42. The inlets and drains on the probe are labeled with arrows on the side. The labels on the tubing set point in the same direction.

Figure 42: Filling the probe with buffer solution

Figure 43: Sealing the probe prior to sterilization

a) Prior to sterilization, fill the dialysis probe with buffer solution using the single-use syringe and the Luer UNF adapter (Figure 42). At the same time, the probe can be checked for any leaks.
b) For sterilization, seal the probe by connecting the Luer connections (Figure 43). This will prevent one-sided overpressure from occurring at the membrane in the dialysis probe, which could damage the membrane.
c) Now sterilize the reactor.
d) After sterilization: Re-open the Luer connector after cooling.
e) As before, fill the probe with buffer solution by using the single-use syringe and carefully check if it flows freely (see Figure 42).
f) Now, the dialysis probe can be connected to the corresponding tubing set of the online analyzer.

6.2.6.4 Connecting the Dialysis Probe to the Tubing Set

The dialysis probe is connected to the online analyzers through the dialysis tubing set. The inlets and drains on the probe are labeled with arrows on the side. The labels on the tubing set face the same direction, see the following Figures 44 and 45.

Figure 44, 45: Connecting the dialysis probe to the tubing set

When starting up the probe after sterilization, remove any air bubbles from the measuring chamber at the online analyzer.

6.2.6.5 Cleaning the Dialysis Probe and Replacing the Membrane

a) Before removing the dialysis probe from the bioreactor, the tubing set is removed from the probe by removing the tube ends.
b) After harvesting the bioreactor or any dead autoclaving phase, remove the dialysis probe from the reactor. The subsequent cleaning of the probe and the adapter is carried out with a soft brush and water.
c) Check to make sure that the gaskets on the dialysis probe adapter are clean and seated properly.
d) Remove the two Torx screws on the end of the dialysis probe using the Torx wrench supplied; this allows the dialysis probe optic to be removed from the optic holder along with the dialysis membrane.
e) Afterwards, clean the probe optic and the optic holder with a soft brush and water.

6.2.7 Connecting the Filtration Probe

The filtration probe (in-line sampling probe) is intended for harvesting microbe-free filtrate from bioreactors and fermenters under sterile conditions. A tubular microfiltration membrane made of polypropylene, positioned inside the bioreactor using an in-line probe, serves as a sterile barrier. The filtration probe is sterilized together with the bioreactor.

The sampling probe is installed in the reactor port according to METTLER-TOLEDO® (INGOLD) standard (12 and 19 mm cover port as well as 25 mm side port). The microbe-free filtrate is sampled continuously or discontinuously at the probe head that is accessible outside the bioreactor | fermenter. For pumping the filtrate, the probe is connected to the filtration tubing set.

Figure 46 below shows the filtration probe for installation in the 12 mm cover port and with stopcock for installation in the 25 mm side port.

Figure 46: Filtration probes in 12 mm and 25 mm design

When assembling and operating the filtration probe, it is imperative that you follow the enclosed instructions.
It is also extremely important that you follow the procedures described for hydrophilizing the polypropylene membrane for installation into the bioreactor, leak testing, in-line sterilization and cleaning of the filtration probe.

6.2.7.1 Description of Setup

The filtration probe for the installation into a 12 mm cover port consists of the filtration unit (1) with membrane support (2), cap nut (3), Ferrule (4), O-ring (5), polypropylene membrane (6), assembly kit (clamping device with friction washer and O-ring) (7) and dummy plugs (8).

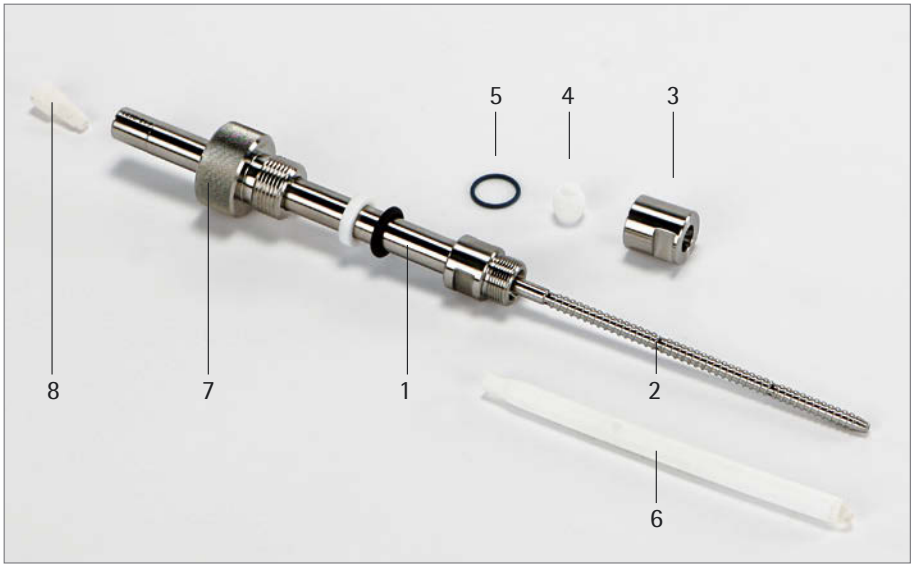

Figure 47: Components of the filtration probe for installation into the 12 mm cover port with PG 13.5 connection

The polypropylene membrane (6) is fastened to the membrane support (2), a threaded pipe made of stainless steel. The permeate created by the membrane is transported through a coil-like channel to a total of 4 cross-drilled holes. They guide the filtrate along the shortest way possible to the collective drill hole of the filtration unit, so that a remixing of the sample can be avoided.

The PTFE Ferrule (4) covers the end of the membrane and the disclosed part of the membrane support. By tightening the cap nut (3), the Ferrule is deformed by pressure and provides a safe seal for the membrane-end facing the membrane support.

The probe thread is protected against the intrusion of microorganisms by the O-ring (5) and by tightening as far as possible.

Depending on the respective installation with the assembly kit's clamping device (7), the immersion depth of the filtration probe varies from 160 to 330 mm.

The dummy plugs (8) are used to seal the filtration probe during sterilization.

6.2.7.2 Optional Accessories

Two 19/12 mm reduction adapters (M26×1 with fine thread and RD 28×1⁄8" with coarse thread) are available for the installation of the filtration probe into the 19 mm cover port.

Figure 48: Filtration probe with adapter M26×1

Figure 49: Filtration probe with adapter RD 28×1⁄8"

6.2.7.3 Assembling the Filtration Probe
Push the Ferrule with the narrow opening first over the membrane support into the conical lower membrane retainer of the filtration unit (Figure 50).

The polypropylene membrane is removed from the packaging and carefully inserted until it stops into the membrane support in the already assembled PTFE Ferrule.

Figure 50: Placing the Ferrule

Figure 51: Attaching the polypropylene membrane

Pull the O-ring over the outer diameter of the filtration unit. Now, the cap nut is carefully pulled over the inserted membrane and screwed into the filtration unit. The screw connection is tightened using 2 open-jawed wrenches until it reaches its mechanical stop position.

Figure 52: Inserting the O-ring

Figure 53: Tightening the cap nut

Moisten the outer diameter of the filtration probe with demineralized water prior to assembly.
The screw connection of the filtration probe is tightened until it reaches its mechanical stop position.

6.2.7.4 Hydrophilizing the Polypropylene Membrane
New polypropylene membranes are impermeable for aqueous media (hydrophobic).

Thus, the membrane must be hydrophilized after installation into the filtration probe. Isopropanol (70%, v/v) is used as hydrophilization solution.

The probe is immersed into the hydrophilization solution inside an upright cylinder of sufficient length that the membrane is entirely surrounded by liquid. The alcohol is pumped through the probe into the closed system with a flow rate of 1–2 ml/min by using a peristaltic pump connected to the sampling outlet of the probe. The hydrophilizing period lasts at least 2 hours. The probe can also be hydrophilized overnight without using the pump by storing it in 70% (v/v) isopropanol.

6.2.7.5 Leak Test
Once hydrophilization has been completed, a leak test is carried out. In order to do this, the hydrophilization setup described in point 6.2.7.6.2 is used.

Instead of drawing isopropanol through the membrane of the probe, air is now pumped into the probe. The flow rate should be equal to the flow rate of the previous hydrophilization.
- Many small air bubbles should start to gradually escape from the membrane. Otherwise, the membrane is hydrophilized insufficiently.
- The escape of large air bubbles from the screw connections is an indication for damaged gaskets.

In order to maintain the hydrophilic properties of the membrane until its installation into the bioreactor, it is recommended to store the membrane in a hydrophilization solution (isopropanol 70%). Immediately before installation into the bioreactor, the isopropanol should be replaced with water in order to avoid evaporation and the loss of hydrophilization.

6.2.7.6 Using the Hydrophilization Set

The hydrophilization set is an auxiliary device which assists in setting-up operation of the filtration probe. It can be ordered optionally.

The polypropylene membrane used for the filtration probe is impermeable for aqueous media (hydrophobic). Therefore, the membrane must be hydrophilized with 70% isopropanol solution prior to each use. This hydrophilization can be conducted easily with the hydrophilizing set and the BioPAT® Trace.

Figure 54: Hydrophilizing set with pump cassette and tubing lines

6.2.7.6.1 Description of Setup

The hydrophilizing set consists of a pump cassette from which two PTFE tubes descend. There is an UNF fitting with Ferrule (for connection to the probe) at one end of the tube. The other end of the tube has no fitting (see Figure 54).

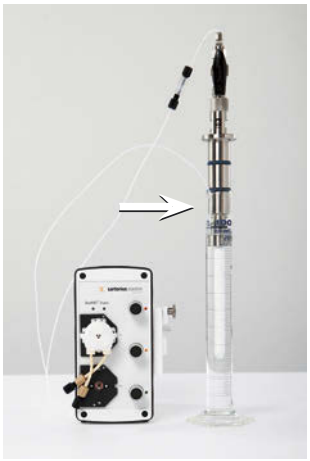

Figure 55: Setup and connection of the hydrophilizing set

6.2.7.6.2 Performing Hydrophilization

After installing the filtration probe, the polypropylene membrane must be hydrophilized with isopropanol (70%, v/v).

The probe is immersed into the 70% isopropanol solution inside an upright cylinder of sufficient length so that the membrane is entirely surrounded by liquid.

The tube end with the UNF fitting is connected to the filtration probe. The free end of the tube is secured in the upright cylinder so that it hangs freely in the air above the liquid level (see label in Figure 55).

The pump cassette of the hydrophilizing set is attached to a motor shaft of the BioPAT® Trace. The second motor shaft remains unused.

As shown in Figure 56, the start window appears after starting the "TraceMon" program in the filtration mode. The operating panel with control buttons for starting (Pump on) and stopping (Pump off) the pump, as well as a control button for the leak test are on the right half of the user interface.

The hydrophilization process is started by pressing "Pump on". The isopropanol solution is sucked through the filtration probe and then pumped back into the upright cylinder. The hydrophilizing period should last for at least 2 hours in order to achieve sufficiently high filtration during operation. When the hydrophilization process is complete, the pump is stopped by pressing the "Pump off" button.

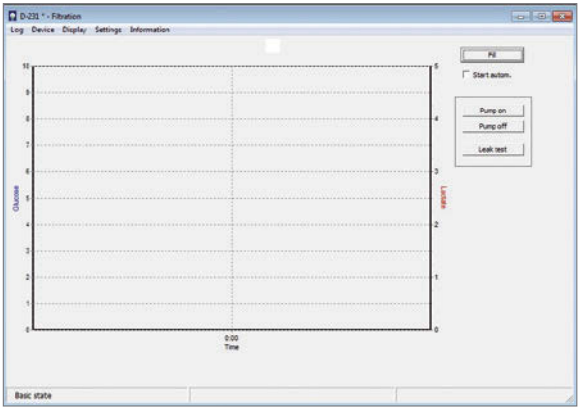

Figure 56: Filtration start window with operating panel to control the pump for hydrophilizing

6.2.7.6.3 Performing the Leak Test
Once hydrophilization has been completed, a leak test must be performed via the user interface (see Figure 56). To this end, the "Leak test" control button is activated.

The hydrophilization setup depicted in Figure 55 is used for the leak test. The free tube end of the hydrophilizing set must hang freely in the air above the liquid level in the upright cylinder (see label in Figure 55).

The test process takes 8 minutes. Meanwhile, air is pumped into the probe. Afterwards, the pump automatically draws the isopropanol back in through the membrane to ensure that the membrane does not become hydrophobic again.

During the test process, the following should be observable:
- After approx. 6 minutes, many small air bubbles should gradually start to escape from the membrane. Otherwise, the membrane is hydrophilized insufficiently.
- If you observe large air bubbles escaping from the screw connections, this indicates that the gaskets are damaged.

In order to maintain the hydrophilic properties of the membrane until its installation in the bioreactor, it is recommended to store the membrane in 70% isopropanol solution.

Immediately before installation into the bioreactor, the isopropanol must be replaced by water in order to avoid evaporation and the loss of hydrophilization. To do so, replace the solution in the upright cylinder (see Figure 55) with water and start the pump at

the operating panel (see Figure 56) by pressing the "Pump on" button. Stop the process after approx. 10 minutes by pressing the "Pump off" button. Remove the hydrophilizing set from the filtration probe and install the probe into the bioreactor according to the operating instructions.

Make sure to observe the instructions regarding the filtration probe in the operating instructions. Improper assembly, pre-treatment or cleaning of the membrane may cause measurement errors or contaminations of the bioreactor!

6.2.7.6.4 Performing Filtration Probe Regeneration after Fermentation

The filtration probe must be removed from the reactor port immediately after drainage and sterilization of the bioreactor. We recommend replacing the membrane and PTFE Ferrule after each use.

In most cases, however, repeated use of the membrane (3 – 5 times) is possible after previous cleaning. In this case proceed as follows:

Remove all deposits attached to the filtration probe, e.g. by using a soft brush under running water.

Next, a weak base (e.g. 0.5 N NaOH) for removal of the protein contamination on the membrane is pumped through the filtration probe into the closed system in filtrate direction; the cleaning cycle lasts approximately 4 hours. To this end, you can use the same equipment as for hydrophilization.

Once the cleaning process is completed, fill the upright cylinder with demineralized water and carefully rinse the filtration probe with the connected hydrophilizing set so that the residues from the alkaline solution are removed.

In order to maintain hydrophilization of the membrane, the probe must be stored in hydrophilization solution (70% isopropanol) until its next use.

If the filtrate flux of the filtration probe is insufficient despite intensive rinsing with alkaline solution, a new membrane with the required PTFE Ferrule must be installed.

6.2.7.7 Installation in the Bioreactor

Prior to each installation of the filtration probe in the bioreactor's extraction ports, all screw connections have to be checked and retightened, if necessary. The filtration probe O-ring must be checked for damage and, if necessary, replaced as well.

Choose a port on the reactor that allows the probe to be positioned in an area with the greatest possible turbulence. A high crossflow rate in the area of the filter surface leads to an extension of the filtration probe service life (crossflow effect).

After installation, make sure that the probe never comes in contact with moving parts (e.g. stirrer, stirrer shaft) inside the bioreactor.

The polypropylene membrane always must be entirely immersed in the liquid during operation of the bioreactor. Otherwise, the filtrate may be drastically decreased.

High hydrostatic pressure at the dispensing location can improve the performance of the filtration unit.

The filtration probe must always be installed immediately before filling the bioreactor, since drying out of the membrane results in a loss of hydrophilic properties.

6.2.7.8 In-line Sterilization of the Filtration Probe

After installation, the filtration probe is sealed with a dummy plug and sterilized together with the bioreactor. During the entire process, the probe must be completely covered with liquid.

Once sterilization is complete and the max. operating temperature of the reactor of 40 °C is reached, the filtrate outlet must be opened and the filtration probe is connected to the BioPAT® Trace via a tubing set for sampling.

6.2.7.9 Connecting the Filtration Probe to the Tubing Set

The filtration probe is connected to the online analyzers through the filtration tubing set.

An adapter cable (Luer UNF) for connecting the filtration probe to the tubing set of the online analyzer BioPAT® Trace is provided with the accessories. As shown in the following Figures 57 and 58, it must be assembled between the probe (UNF connection) and the BioPAT® Trace tubing set (Luer connection).

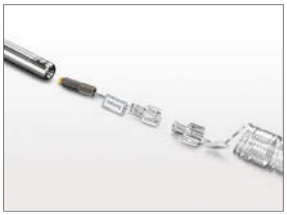

Figure 57: Filtration probe with tubing set

Figure 58: Connecting the tubing set

6.3 Loading the Software and Network Connection

The BioPAT® Trace is run internally by a computer and can be operated as a full-ranked member in intranets and LANs, or can be directly interfaced with a PC.

In order to do this, the operating software supplied must be installed on the designated PC.

The files needed for the installation of the operating software trace_mon are stored in the device's data memory and can be accessed via an internal device website.

To install the software, proceed as follows:

Connect the BioPAT® Trace to your network and navigate to the factory-set IP address 192.168.120.231 in your web browser.

Here you have the option of downloading important files and the operating instructions onto your computer and of assigning the device an IP address appropriate for your own network. If you do not want to make any changes (if there is a direct connection with a PC via a cross-over Ethernet cable, for example), then the device will work with the factory-set IP address. Make sure that your PC is not set to automatic address assignment when using a direct connection!

First download the other important files for the rest of the installation onto your computer by clicking the right mouse button. They can be found in the "Download" menu item. The following files can be found here:

- setup_tracemon_x_x_x as setup file of the operating software (_x_x_x = version number)
- ip_set as the backup file of the boot file
- Operating instructions for the BioPAT® Trace as PDF file

Then change the device's network address. It can be found in the "Network settings" menu item.

Figure 59: Login for network settings

In order to do this, you will need to log in with the password **TuSh1Vf5Ja**.

In most cases, the factory-set IP address will not be compatible with company networks. Have a suitable IP address assigned by your network administrator; otherwise the device may not be able to connect with the operating software.

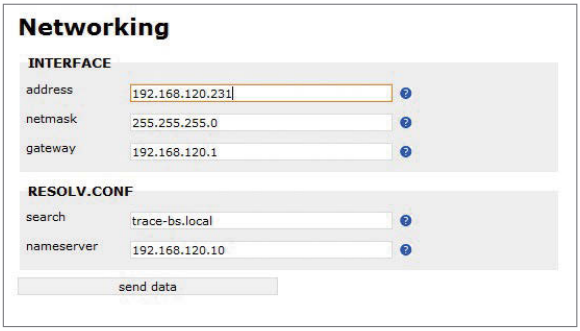

Figure 60: Input field for network settings

If you would like to work via a LAN or intranet, that the factory-set network address or the network name may not be compatible with your network. If this is the case, please contact your network administrator and change the BioPAT® Trace's IP address in the form field.

After the network address has been changed, the BioPAT® Trace can only be accessed via this new IP address. The last 3 characters of the IP address (here 231) represent the number of the device that is displayed in the user interface.

6.3.1 Troubleshooting

If you have entered different or incorrect IP addresses on the device website and during the installation, it will not be possible to establish a connection. The following options are available:

First, attempt to produce a connection to the internal device website via the standard IP address 192.168.120.231 using a direct PC connection and a cross-over Ethernet cable.

If this is not possible, a change to a known IP address must be forced using the boot file. First switch the device off. Then copy the boot file “ip_set” onto a USB stick and connect it to the back of the device. Then switch the device on. Once the boot process is complete, the device will now have the new IP address 192.168.120.232.

Navigate to the new IP address 192.168.120.232 in your web browser.
After the device has been connected in this manner, you can set the network address via your web browser as described above.

6.4 Installing the PC Software

Run the file "setup_tracemon" to install the operating software on your computer. When doing so, you will be asked to enter the new IP address. In order to do this, overwrite the suggested factory-set IP address. If you have not changed the IP address then use "Next" to confirm.

During the installation you will be asked to specify the storage locations for the program and the subsequent measurement data. Next, the software will start the device with the specified IP address and automatically connect to the BioPAT® Trace.

6.5 Changing the Start Settings

If you change the IP address at a later point in time, you should also update the start settings in the program link of the "Start" icon to make sure that the device still connects automatically. To do this, complete the following steps:

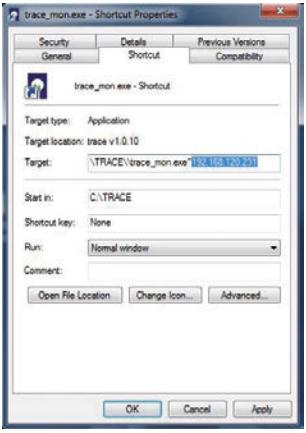

Figure 61: Start settings

1. Switch the screen view to your desktop.
2. Click on the start icon with the right mouse button.
3. Select the menu item "Properties".
4. Enter your new IP address at the place indicated in the adjacent figure 61.
5. Confirm the change with "Apply" and then "OK"

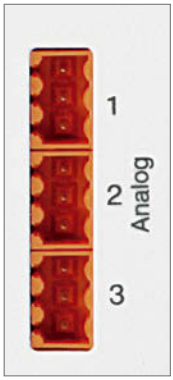

Figure 62: Analog outputs

6.6 Assignment of Analog Outputs

The measured values remain available for further use as analog signals at the ports analog 1 (Measurement channel 1 – Glucose) and analog 2 (Measurement channel 2 – Lactate). When using the ethanol | methanol method, the signal is output on analog 1. The output analog 3 is not assigned.

1-1	0 (2) – 10 V	2-1	0 (2) – 10 V
1-2	GND	2-2	GND
1-3	0 (4) – 20 mA	2-3	0 (4) – 20 mA

In the "Parameters" menu (7.1.4.1.), the output format can be switched between 0 – 10 V and 2 – 10 V or 0 – 20 mA and 4 – 20 mA. The output ranges of the analog outputs are also configured here.

The output ranges of the analog outputs can be configured as described in Chapter 7.1.4.1 "Parameters" on page 102.

The signal at the analog output is changed whenever there is a new measurement value and remains unchanged unless:
a) there is a new measurement value,
b) the device is rebooted,
c) "new tubing set" is activated.

6.7 Device Startup

Before every startup, check the BioPAT® Trace to make sure that all modules installed on the device are functional. The following conditions must be checked scrupulously on the device:

- Are the two original pump cassettes installed properly?
- Is the tubing set connected completely and properly?
- Is the enzyme reactor installed in the methanol | ethanol operating mode?
- Is there a measurement cell in the holder?
- Are the available volumes of transport buffer and the appropriate calibration solutions ready at hand?

If these checks are passed, you can start the device. Chapter ▶ "7. Operation" on page 85 describes in detail the operating software functions necessary to start the device. In principle, the following procedure must be maintained:

1) In the "Basic settings" menu, select the measuring method (Chapter ▶ "7.1.4.2 Basic Settings" on page 106).
2) In the "Parameter" menu, configure the calibration standards and measuring and calibration frequency you want to use (Chapter ▶ "7.1.4.1 Parameters" on page 102).
3) After you have connected all tubing and sampling lines, start the filling routine (Chapter ▶ "7. Operation" on page 85).
4) After priming, the device starts with the measurements automatically (if the autostart option was activated) or switches to the "Standby" mode.

If you are working with the dialysis method, you must carry out a reference measurement at the right time in order to obtain conclusive results. Proceed as described in Chapter ▶ "7. Operation" on page 85.

6.8 Decommissioning

The device can be switched off at any time by pressing the power switch. The calibration and reference values last stored remain in memory for the next start (see Section ▶ "7.1.2.1 Connect" on page 93).

If the measuring instrument is not operated for two days (e.g. over the weekend), the buffer-filled system may remain standing. In the event that the device will be out of use for longer periods of time, the tubing set must be replaced before re-starting operations.

For decommissioning in the methanol | ethanol operating mode, the enzyme reactor must be removed and stored at a cool temperature (see Chapter 6.2.5 Connection and Removal of the Enzyme Reactor).

7. Operation

Switch on the BioPAT® Trace at the power switch located on the back panel and run it on an external PC.
You will find the start icon on the PC's desktop . Double-clicking it will start the application. The user interface shown in Figure 63 appears.

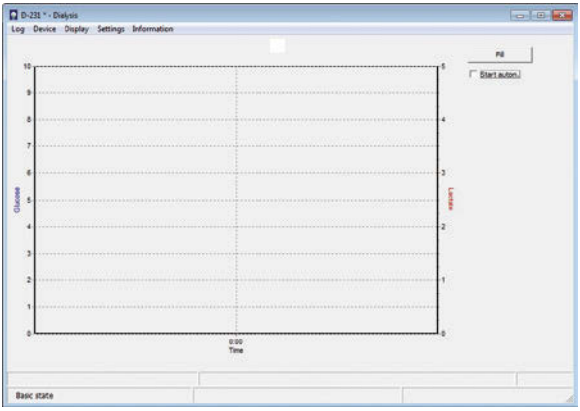

Figure 63: Start window indicating the selection and display ranges

The device number appears in the header. In this case, it's 231*. The asterisk indicates that a connection exists between the control PC and the BioPAT® Trace. If there is no connection to the measuring instrument, it must be established (see Section ▶ "6.3.1 Ethernet Connection" on page 80).

The following menu items can be selected from the task bar: File, Device, View, Settings and Information.

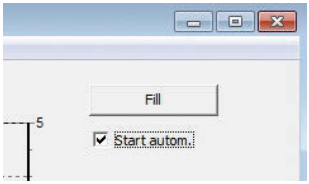

Figure 64: Autostart

In the measuring window in the center of the screen, the glucose and lactate measurements are plotted over time. The glucose values are represented by blue dots and the lactate values by red dots.

The device status is displayed under the measurement window. At the beginning, the device is in default mode. To the right of the measuring window there is an area for context-based selection buttons.

Every time the device restarts, completely fill the tubing set and run several preliminary measurements to render the sensor system stable before initiating the calibration routine. This process is called priming; alongside filling and preliminary measurements, it also includes an initial calibration of the system. After priming, the device switches to "Standby" mode. Depending on the measuring method selected, the entire process takes between 40 and 60 minutes.

Another option is to configure the BioPAT® Trace to start with the measurements immediately after the priming routine. If a check mark is placed next to "Start automatically" (Figure 64), the device will automatically initiate the measurement after performing the initial calibration routine.

If the "Fill" button is pressed, the device starts filling and the "Fill" button disappears.

Before you startup your system with autostart, make sure that the tubing set is properly connected to the sampling outlet on the bioreactor.

When the system is ready to run the measurement, the line on the lower right will appear (Figure 65). In filtration mode, two buttons become available after measurement ready mode has been achieved. The one is the "calibration" button, which can be pressed to run any new calibration measurement with the device, and the other is the "Start" button" that activates the measuring routine.

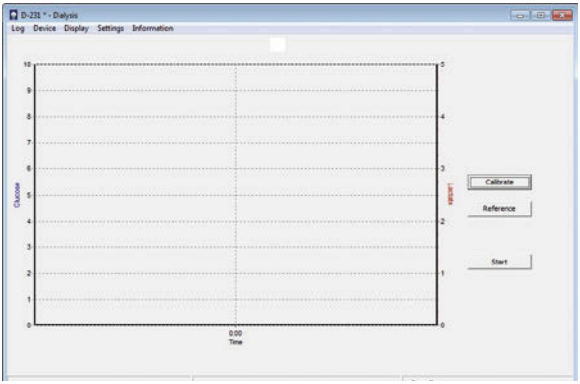

Figure 65: Screen display in "Standby" mode

In the dialysis mode, a third button labeled "Reference" appears to the right of the measuring window. If the "Reference" button is activated, the device starts with a reference measurement to calibrate the attached dialysis probe.

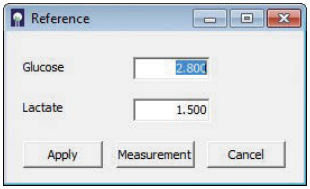

Figure 66: Display after reference measurement

Reference (for dialysis only)
A reference measurement must be performed to compare the permeability of the two diffusion membranes in the diffusion cell (that is only allowed contact with calibration solutions) and in the dialysis probe (that only has contact with the reactor contents). The relationship of the two membranes to one another is expressed by a reference factor that is used for calculating the measured values.

You can conduct a reference measurement whenever you want, in particular when changes occur in the process that may influence the diffusion speed (e.g. as a result of temperature changes).

Reference measurements can also be carried out at regular intervals, e.g. when samples are taken for external accompanying analysis. The measured value is only corrected with a new reference factor when the new reference value was entered and confirmed by pressing "Apply". Up to then, measured values that were determined in the meantime are always calculated and displayed with the current reference factor.

The device starts with a reference factor of 1.0.
If the two membranes were permeable to the same degree, then this value would be correct. In practice, however, experience has shown that manufacturing-related and ambient conditions affecting the probe can result in differences.

The comparison is activated as follows: After clicking the "Reference" button, the device determines the concentration in the reactor and suggests the corresponding values (see Figure 66).

Now, using a reference sample, you must check whether these values are correct and adjust them accordingly. If you already know the concentration values (e.g. from the initial weight), you can enter these values directly. Alternatively, you can determine the concentrations in the reactor with an external sample using an off-line analyzer.

Confirm the prompted measured values with "Accept". If you want to make changes, enter the new values into the entry field (with decimal point) and confirm with "Accept".

With the "Discard" function, you can cancel the procedure and move back to the starting position.

As long as the input window is open and no new reference values were entered and confirmed, the BioPAT® Trace continues working with the current reference factor.

If determining a reference measurement using an external analyzer is going to take longer and the medium concentration is expected to change in the meantime, you should wait for the reference measurement and only then take a sample for external analysis. When the input window for the reference values appears, the device has stored the currently measured concentration to compare it with subsequently entered values and determine the new reference factor.

If no off-line analyzer is available for determining reference values, you can also measure the sample on the BioPAT® Trace. To do so, select the "Measurement" function and follow the further instructions prompted by the menu navigation (see Figure 67).

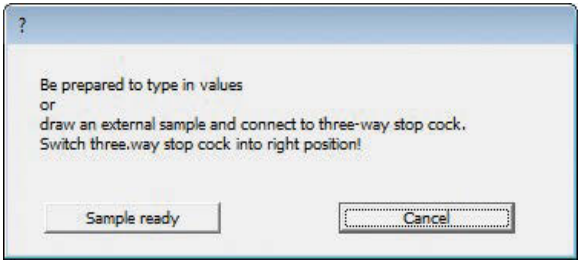
?
Be prepared to type in values
or
draw an external sample and connect to three-way stop cock.
Switch three.way stop cock into right position!
Sample ready
Cancel

Figure 67: Prompt for sampling and connection to the three-way stopcock

Draw a cell-free sample from the bioreactor (at least 5 mL) into a 5 mL syringe and connect it to the three-way stopcock as shown in Figure 68. Check that the three-way stopcock is in the correct position:
The arrows on the stopcock must point towards the green tube marking and the syringe.

Figure 68: Connecting the external sample

Only use the "Sample ready" function if you are sure that the filled syringe is connected and the three-way stopcock is set to the proper position. Otherwise erroneous measurements may result!

"Sample ready" is activated to measure the external sample. Three measurements are taken and their average displayed as a result. As described above, you can then accept, correct or discard measured values or you can perform another measurement on an external sample.

Figure 69: Correct position of the three-way stopcock

Before you continue working, make sure that the three-way valve was set back to the initial position (see Figure 69) so that you can correctly measure Standard 1 during the next calibration.

Make sure that the three-way stopcock is set as displayed. Otherwise all following calibrations will be erroneous and the resulting measurements incorrect!

7.1 Menu Bar

7.1.1 Menu Item “Log”

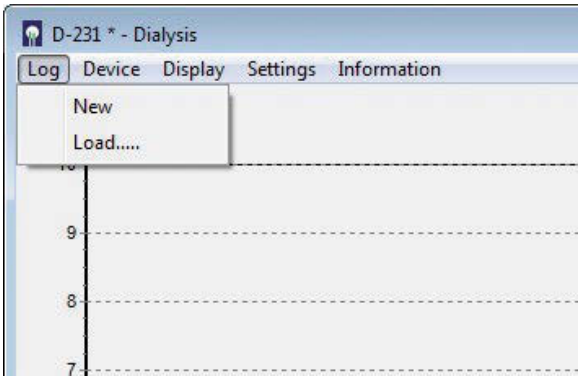

Figure 70: “Log” menu options

The measured data are recorded as text in a ptd file and saved in the default subdirectory “Data”. If you want to create a new file, select the menu item “New”.

If you want to load and display measured data from an old file, select the menu item “Load...”.

The saved files can be easily processed, e.g. in Excel. For notes on importing as an Excel file see Chapter 8.5.

7.1.2 Menu Item “Device”

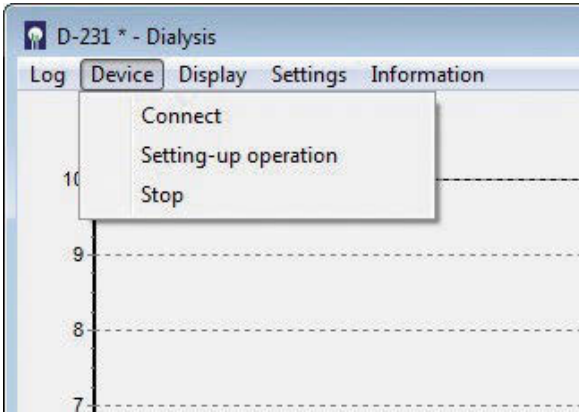

Figure 71: “Device” menu options

The user can choose from three sub-items: “Connect”, “Setting-up operation” and “Stop” (see Figure 71).

7.1.2.1 Connect

By selecting the “Connect” menu item, you have the option to connect a new BioPAT® Trace (Figure 72). To do so, you must enter the IP address of the device.

If you have started the software by clicking on the start icon on the desktop, the standard IP address 192.168.120.231 will already be displayed.

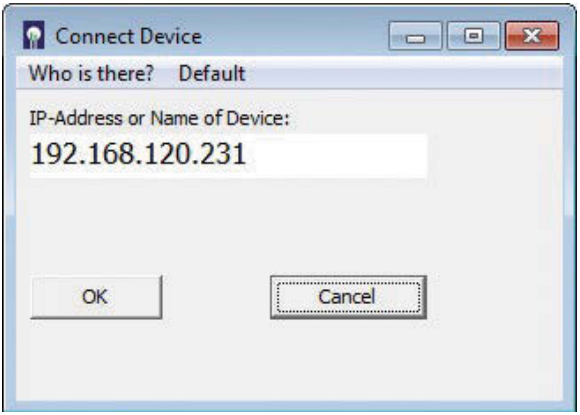

Figure 72: Connect device

If your device has been assigned a different IP address (e.g. by your network administrator), then enter that address here.

If you would also like to be prompted with this new address automatically in the future, proceed as described in Section ▶ "6.5 Changing the Start Settings" on page 81.

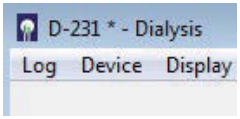

Figure 73:
Connection established

Once you have entered the correct IP address and the device is acknowledged by the network, it will connect to the control PC. An asterisk appears in the view bar behind the name or number of the device.

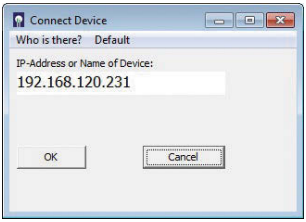

Figure 74: Searching the network

Alternatively, you can start a search for new devices on the network by clicking on the button "Who is out there?". The results will be displayed after a little while. You can highlight the desired device and select it with "Ok".

If no device can be found, an error message appears. Try to enter the IP address directly or contact your network administrator.

7.1.2.2 Setting-up operation

If the tubing set has been replaced, you must go to to the menu item "Setting-up operation". "Setting-up operation" deactivates all previous calibration and reference data and requires that the tubing system is re-primed.

7.1.2.3 Stop

"Stop" is used for temporary decommissioning of the system, e.g. when the monitored bioprocess is interrupted, for short operating interruptions or when connecting enzyme reactors (see Chapter 6.2.5, page 52). Select the menu item "Stop" to interrupt the measuring routine and ready status. All pumps stop and the valves shut. In this event, the device will retain stored data on calibration and reference values.

Click the “Stop” button and the BioPAT® Trace switches to standby. The program can now be closed. The device can also be switched off at the power switch without losing any data. When switching the device back on or re-booting the control program, the prompt screen shown in Figure 75 appears.

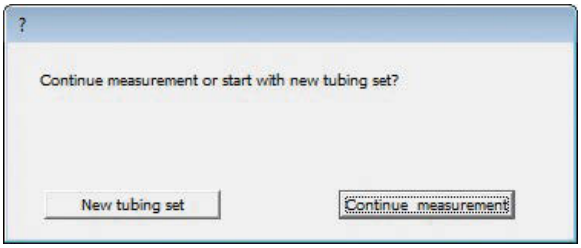

Figure 75: Restart prompt window after “Stop”

By clicking on the button “Continue measurement”, the system switches directly to operational mode. No rinsing cycles or calibrations will be carried out. The former calibration values will be retained.

Do not activate “Continue measurement” unless you are certain that the same conditions prevail as before the pause (e.g. in the event of brief interruptions). Otherwise, this may result in erroneous measurements!

In the case of longer interruptions (e.g. overnight), it is recommended to carry out a calibration before continuing with further measurements.

7.1.3 Menu Item “Display”

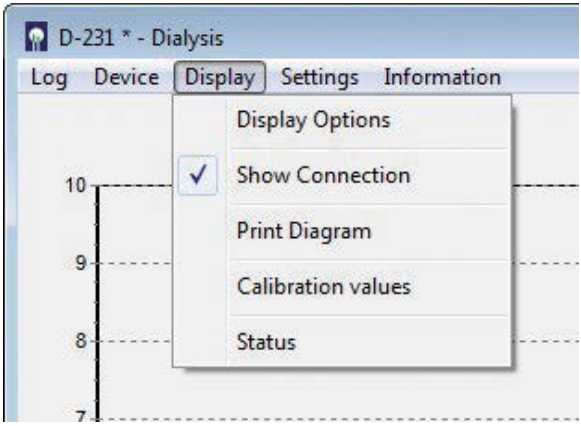

Figure 76: “Display” menu options

When the menu item “Show connection” is activated, an asterisk indicates that the control PC is communicating properly with the BioPAT® Trace.

By selecting the menu item “Print diagram”, the currently displayed diagram is printed out on an interfaced printer.

When the menu item “Calibration values” is selected, a window opens that shows the current calibration values (Figure 77).

The axis intercepts (a0), calibration gradient (a1) and the reference factors (Reference fr) are displayed.

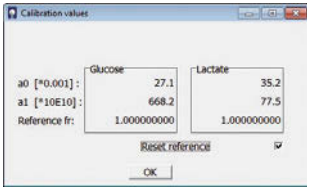

Figure 77: Screen shot of current calibration values

Activating the option “Reset reference” resets the reference factors to the factor 1.0 (only in dialysis mode).

When the menu item “Status” is selected, an information bar appears at the bottom edge of the screen that displays further information on the current status in three fields (see Figure 78). This function is of particular interest for long intervals or group measurements. The number of pending group measurements (or preliminary measurements) is displayed in field 1. In field 2, a current time count counts down the time until the end of the current action (in seconds). The remaining time in the interval of the measurement period is displayed in field 3 (in seconds).

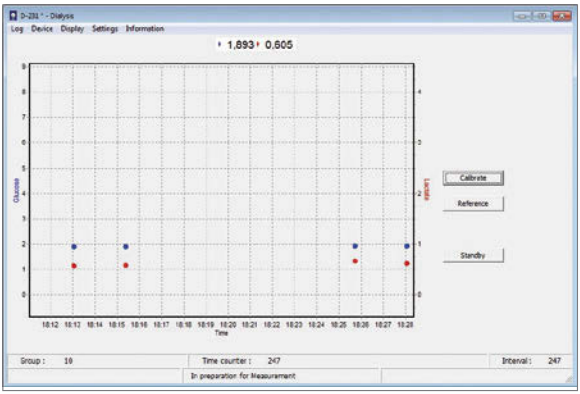

Figure 78: Screen display of status bar

7.1.3.1 Change View

The presentation of the diagram can be modified in the menu item “Change view”. Here, you can configure dot sizes, dot colors and the axis scales (Figure 79). The default settings are the color blue for glucose and red for lactate. A green dot color is preset for methanol or ethanol.

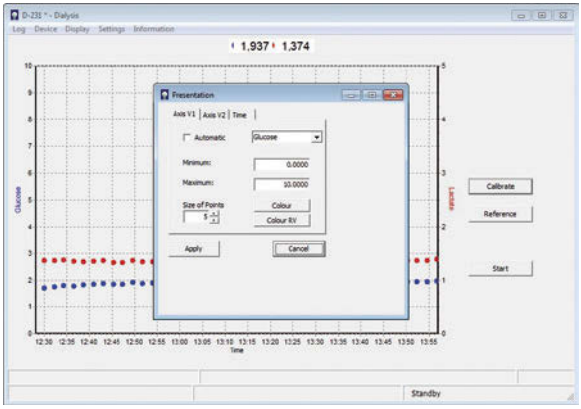

Figure 79: Screen shot for adjusting the measured values display

The measurement display can be modified at any time during operation. Press the right mouse button to display the input field for configuring the view (Figure 80).

The following additional options can be selected:
- Moving the graph:
  Hold down the right mouse button and move
- Zoom:
  Pull the window from the upper right down to the lower left (hold down left mouse button).
- Return to Start screen:
  Pull the window from the lower left up to the upper right (hold down left mouse button).

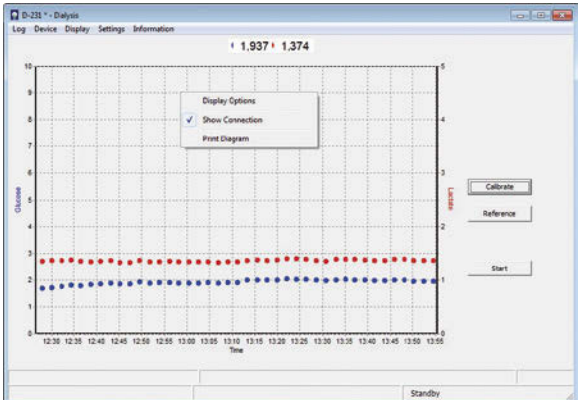

Figure 80: Display of the measurement curves

7.1.4 Menu Item “Settings”

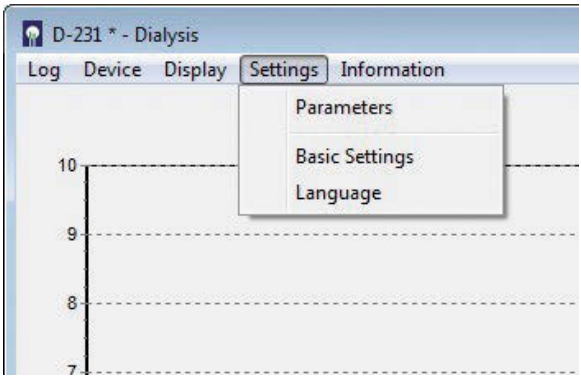

Figure 81: “Settings” menu options

In the “Settings” menu options, you can configure the most important device settings for operation and communication:

– Parameters
  allow the user-specific configuration of measurement frequency, calibration frequency, concentration of the calibration standards etc.
– Basic settings
  Here you can configure the device for the selected sampling method. Likewise, the user can enter information about the device and the project that later appear on the data output.
– Language
  Here you can change the menu guidance language.

7.1.4.1 Parameters

All relevant parameters for measuring operations can be configured in the “Parameters” sub-item (Figure 82).

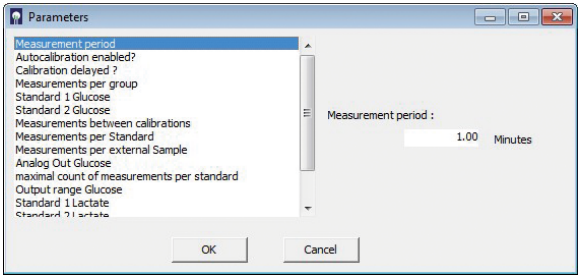

Figure 82: Entry screen for configuring the measurement parameters

- Measurement period
  - Enter the desired measuring interval here. When the value 1 is entered, a measurement is performed every minute or at the maximum frequency. If one analysis should be performed every hour, then enter 60.
  - The frequency can be changed at any time and becomes immediately effective by clicking “OK”.

- Autocalibration enabled?
  Select yes|no. If you allow autocalibration, recalibration takes place automatically after every × number of measurements (see: measurements between calibrations).

- Calibration delayed?
  Normally, a calibration is performed after a certain number of measurements. Longer interruptions (e.g. measurements every 6 hours for cell cultivations) can lead to very long periods expiring between the most recent calibration and the next measurement.

Activating the delayed calibration function schedules the calibration to be performed shortly before the next measurement. This way, an up-to-date calibration is available.

– Number of measured values in groups
When using larger measuring intervals, it can make sense to run several measurements with maximum frequency in quick sequence at a given point of time. This way, for example, a group of 10 measurements may be carried out every 6 hours. The number of measured values in the group can be set here. No average is calculated. Every measured value is displayed immediately after measurement. If the time required for the performance of the group is longer than the measurement period, then the next group starts with the new measuring interval.

For the determination of the number of measurements between calibrations, every measurement in a group is taken into account. This must be given due consideration when selecting the calibration settings!

– Standard levels (glucose and lactate)
Here, you indicate the concentrations of the calibration standard solutions that have been selected and hooked-up for your application.

– Measurements between calibrations
Here, you can enter how many measurements should be performed before an automatic calibration is run. This feature only works, however, if the autocalibration function has been allowed.

By pressing the "Calibration" button, you can initiate a calibration at any time.

Any changes made to the automatic calibration data during running operations are not valid until the next calibration.

- Measurements per standard
  This function is used to set the number of measurements per calibration standard. One minute is required for each measurement of a calibration value. That means that a normal calibration with 3 measurements per standard will take 6 minutes (including rinsing times for changing the medium).

- Output range (glucose and lactate)
  Here, the output ranges of the analog outputs are set. Proceed as follows:
  The upper limit of the measuring range is always indicated. The lower limit is preset to 0.

  For a required measurement range of e.g. 0-10 g/L glucose, glucose is set to 10 (upper limit) in the output range. When selecting the voltage range of 0 – 10 V, then 10 volts are equal to 10 g/L glucose.

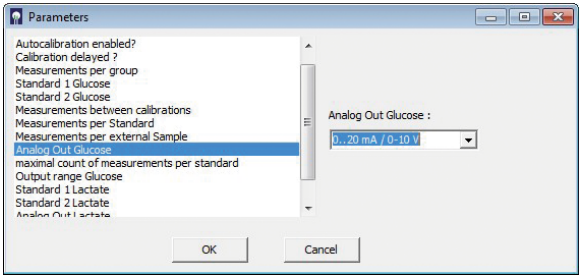

Figure 83: Switching the analog output

Here, you can switch the signals of the analog outputs between 0 – 20 mA and 4 – 20 mA or between 0 – 10 V and 2 – 10 V (see Figure 83).

7.1.4.2 Basic Settings

In the "Basic settings" menu item, you can enter data that is important for carrying out and documenting the measurements (Figure 84).
Before running the measurement, you can enter the project name, device location and user ID.

The most important item is the selection of the application used. You can either select filtration or dialysis for the determination of glucose and lactate as well as methanol | ethanol filtration and methanol | ethanol dialysis.

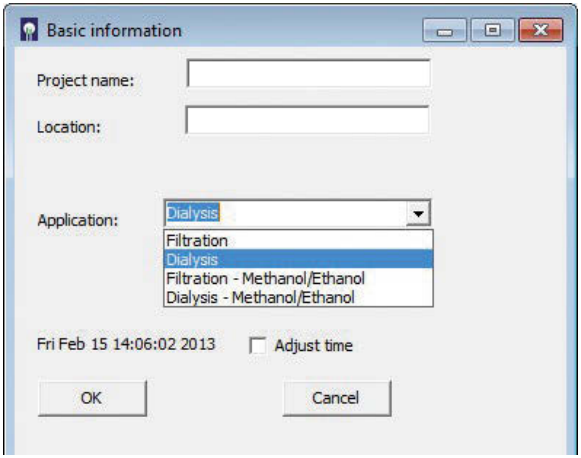

Figure 84: Entry screen for basic settings

Before working with a new tubing set, always check to make sure that the right application has been selected! Otherwise, this can result in erroneous measurements or destruction of the tubing set!

By placing a check mark next to “Adjust time”, you can compare the system times on your PC and the BioPAT® Trace. The BioPAT® Trace will then use the PC's system time.

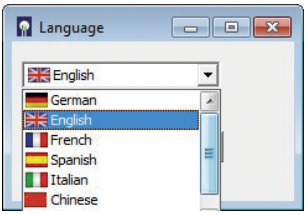

Figure 85: Selection window for language settings

7.1.4.3 Language

You can select your preferred language under the “Language” menu item (Figure 85).

7.1.5 Menu Item “Information”

Select the menu item “Information” to display the current software versions of the BioPAT® Trace and the control software.

7.2 LED Status Messages

In order to be able to read the device status independently of the operating software on a connected PC directly on the BioPAT® Trace, the various operating statuses can be read using the LEDs on the front. The following table 4 provides an overview of the LED signals:

Status	Action	LED1 (green)	LED 2 color	LED 2 status
Setting-up Operation and Decommissioning				
Boot process		on	white	on
Stop		on	cyan	on
Filling		on	cyan	flashes
Preliminary measurement		on	green | cyan	flashes
Measurement				
Measurement	loaded	on	green	on
Measurement	unloaded	on	green	flashes
Breaks in Measuring				
Readiness	readiness	on	yellow	on
Interval	standstill	on	yellow	flashes
Rinsing Cycles				
Changing the medium		on	magenta	on
Rinsing probe		on	magenta	flashes
Calibrations				
Calibration	loaded	on	blue	on
Calibration	unloaded	on	blue	flashes
Reference	loaded	on	blue	on
Reference	unloaded	on	blue	flashes
Faults				
No network connection		on	red	on

Table 6: LED status messages

8. Tips and Checklist

8.1 Choosing Suitable Standard Solutions

Various combinations of calibration standard solutions are available to optimally cover the expected concentration cycles. We recommend connecting a higher concentrated standard than standard 1.

Depending on the process conditions and the objective of the cultivation, various concentration ranges have to be covered. The concentration range should principally be between the concentrations of the standard solutions in order to ensure optimal measuring accuracy. Furthermore, we recommend always choosing a standard with very low concentration in order to properly determine measured values close to zero.

For regulation, the concentrations of the calibration standards should not be too far from the regulation (set)point. For regulation at 1g/L, for example, it is more practical to use a combination of 0.5 g/L and 4 g/L than a combination of 1 g/L and 10 g/L, for example.

8.2 Dialysis Mode: Reference Measurements at the Start of Fermentation

It is crucial to perform reference measurements when using the dialysis method to compare the permeability of the membranes inside the reactor and outside the diffusion cell as well as to compensate for temperature differences between both membranes. Thus, reliable determination of the reference factor is very important for the quality of the measured values.

It is therefore recommended that you determine the reference value as soon as possible at the start of the cultivation and do so in a phase without fluctuations in the concentration of the medium. For this purpose, deactivate autocalibration and then set the measuring interval to 1. Afterwards, track some measured values. If they do not spread too widely and are as constant as possible, you can trigger reference measurement. Once the reference measurements are completed, you should wait for some measurements and check if the recalculated values meet your expectations. If necessary, you can also repeat the reference measurement. As soon as you are satisfied with the result, you can readjust the measurement parameters and start with the cultivation.

8.3 Dialysis Mode: Reference Measurements for Low Lactate Concentrations

In many cases, there is a surplus in glucose and the medium is (still) lactate-free at the start of a cultivation. When performing reference measurements, the BioPAT® Trace suggests a value once the reference measurement is completed. This value is usually overwritten by entering the known value (known due to the media composition or determined with an external analyzer). If the available lactate concentration is still very low, do not yet overwrite this value (the reference factor for lactate is still 1.0). During the course of the cultivation, the increase in lactate concentration can now be observed more easily. In due course, a standard reference measurement overwriting both values can be performed.

8.4 Avoiding Contamination in the Tubing Set

During longer processes (over a period of weeks), biofilm can form inside the tubing set over time. This can result in the lines clogging or in highly fluctuating measured values.
This applies in particular to storage tanks with ready-for-use diluted transport solution that are repeatedly refilled with fresh solution. It is advantageous to completely replace the almost-empty container with a new container with fresh solution to avoid a transfer of microbes.

The rinsing line of the measurement cell when removing air bubbles in the measuring chamber presents another opportunity for microbes to intrude into the tubing set. Therefore, it is imperative to use only fresh solutions and | or a sterile filter attachment for this purpose.

In some long-term cultivations, it was observed that biofilm would form in the waste line and spread into the drip chamber. In this case, it is also recommendable to regularly replace the waste containers and to prevent the waste line from being immersed in the waste. The waste containers can be further protected by adding disinfectant solution (see Consumables Table 3).

When using the filtration method, the disinfectant solution can even be added to the transport buffer (0.5% v/v) directly. This way, the most effective protection against contamination is achieved. However, this procedure cannot be used for the dialysis method since the transport solution penetrates the dialysis membrane and the disinfecting agent can then enter the reactor through the membrane directly.

8.5 Import Data to Excel

The measured values of the BioPAT® Trace are saved on the PC as a ptd file. For further processing, the data can be imported into Excel. The data import of the ptd file into Excel is carried out in seven steps:

1. Open the MS Excel program.

2. Select "File" | "Load"
and select the directory in which the ptd file is saved.

3. Activate "All files"
(circled in orange)

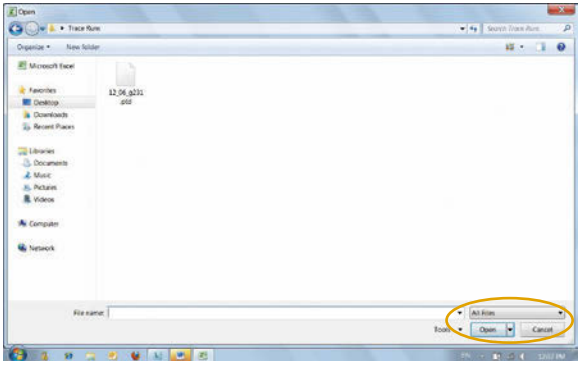

Figure 86: Importing data from the ptd file into Excel. Activate "All files"

4. Highlight the ptd file (circled in orange)
Confirm your selection by pressing the "Load" button (circled in red).

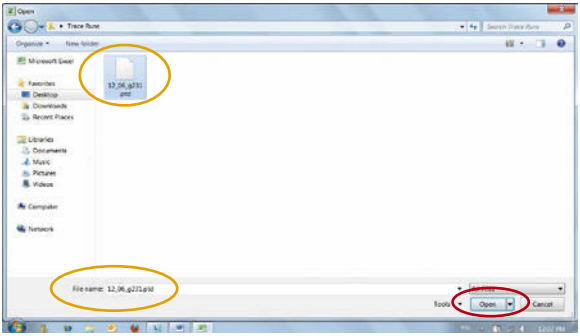

Figure 87: Import data of the ptd file into Excel "Highlighting the ptd file"

5. Text conversion assistant (step 1)

The text conversion assistant will then start. The file is read in three steps. First, select the field "Separated" (circled in orange). This step is completed by pressing the "Next" button (circled in red).

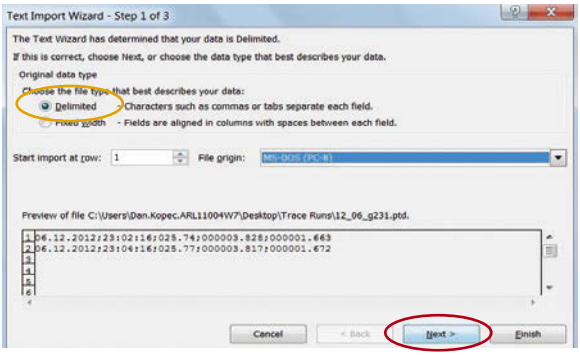
Text Import Wizard - Step 1 of 3
The Text Wizard has determined that your data is Delimited.
If this is correct, choose Next, or choose the data type that best describes your data.
Original data type
Choose the file type that best describes your data:
Delimited - Characters such as commas or tabs separate each field.
Fixed width - Fields are aligned in columns with spaces between each field.
Start import at row: 1
File origin:
Preview of file C:\Users\Don.Kopec.ARL11004W7\Desktop\Trace Runs\12_06_g231.ptd.
1 06.12.2012;23:02:16;025.74;000003.828;000001.663
2 06.12.2012;23:04:16;025.77;000003.817;000001.672
Cancel
< Back
Next >
Finish

Figure 88: Importing data from the ptd file into Excel, text conversion assistant step 1

6. Text conversion assistant (step 2)

In this step, the "Semicolon" separator is selected (circled in orange) and confirmed by pressing the "Next" button (circled in red).

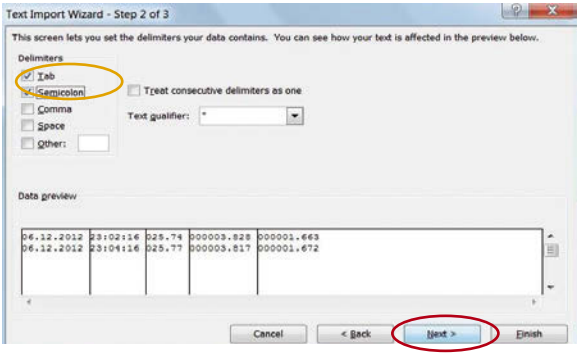

Figure 89: Importing data from the ptd file into Excel, text conversion assistant step 2

7. Text conversion assistant (step 3)

In this step, the "More" option is selected (circled in orange).

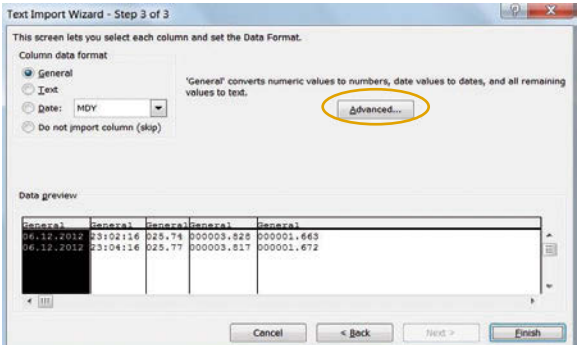
Text Import Wizard - Step 3 of 3
This screen lets you select each column and set the Data Format.
Column data format
General
Text
Date: MDY
Do not import column (skip)
'General' converts numeric values to numbers, date values to dates, and all remaining values to text.
Advanced...
Data preview
General General General General General
06.12.2012 23:02:16 025.74 000003.828 000001.663
06.12.2012 23:04:16 025.77 000003.817 000001.672
Cancel
< Back
Next >
Finish

Figure 90: Importing data from the ptd file into Excel, text conversion assistant step 3

Here you can select “More text import options”. A period “.” is set as the decimal separator and a space as the separator for thousands (circled in red).

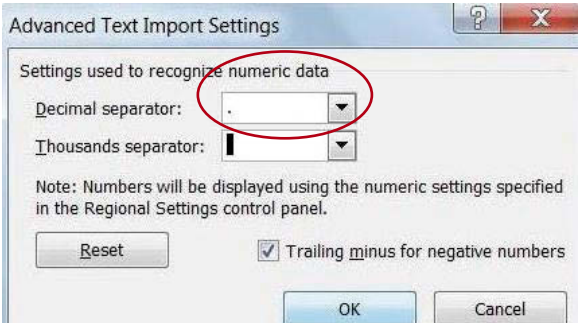

Figure 91: Import data of the ptd file into Excel “More text import options”

After confirming this window by pressing “OK”, confirm step 3 of the text conversion assistant by pressing “Next”. The data from the ptd file are now available as an Excel spreadsheet.

Checklist for Setting-up Operation

Step	Action	Chapter	Page	Figure	Checklist
0	Install PC software (only on initial setup)	6.3 and 6.4	77ff	59 – 61	
	Access website of the device		77	–	
	Download installation program		77ff	–	
	Start installation program		78ff	–	
	Adjust IP address and subnet screen		79	59 – 61	
1	Prepare probes	6.2.6 and 6.2.7	55ff	37 – 58	
	Install membrane		55ff | 64ff	37 – 40 | 50 – 53	
	For filtration only: Hydrophilize membrane with isopropanol		69ff	54 – 56	
	Install probe in reactor (and sterilize, if necessary)		58ff | 74ff	41 – 43 | –	
2	Prepare transport buffer and waste container	6.2.2 and 8.4	44 | 111	–	
	Dilute buffer concentrate and prepare in bottles	6.2.2	42	–	
	Prepare waste container with disinfecting agent, if necessary	8.4	111	–	
3	Setup device tubing	6.2.2 to 6.2.4	42ff	17 – 33	
	Align and slightly open guide plate		45ff	19	
	Insert and pull down valve tubes into valve slot		46	20 – 22	
	Push guide plates in direction of valves for protection		47	23	
	Insert and engage pumps		47ff	24 – 26	
	Place measurement cell into recess and affix with knurled nut		48ff	28 – 29	
	Attach diffusion module		50	30	
	Insert drip counters		50	31	
	For dialysis only: Connect three-way stopcock (optional)		51	32 – 33	

Step	Action	Chapter	Page	Figure	Checklist
4	Connect inlet and outlet lines	6.2.2	44	–	
	Connect tubing to transport buffer		44	–	
	Connect tubing to calibration solutions		44	–	
	Connect waste line to waste container		44	–	
5	Switch on device	7	85	–	
	Switch on power switch on back of device	7	85	–	
	Wait for internal computer to boot (white LED turns red)	7.2	108	–	
6	Start PC software	7	85ff	63 – 73	
	Double-click "Start" icon on desktop (trace-mon)		85	63	
	Wait for automatic connection setup or connect device	7 and 7.1.2.1	85 | 93ff	72	
	Start with selecting "new tubing set"		96	73	
7	Set operating parameters ("Settings" menu)	7.1.4	101ff	79 – 80	
	Basic settings	7.1.4.2	105ff	82	
	Select measuring method (filtration, dialysis)		105ff	82	
	Measurement parameters	7.1.4.1	102ff	80 – 81	
	Set measurement period		102	–	
	Enter calibration standard values		103	–	
	Create file for saving measured values	7.1.1	92	70	
8	Connect sampling probe	6.2.6.4 and 6.2.7.9	60 | 76	44 – 45 | 57 – 58	
	Connect filtration or dialysis probe to tubing		60 | 76	44 – 45 | 57 – 58	
9	Starting the measurements	7	85ff	64 – 65	
	Confirm "Fill" button and wait for calibration		86ff	64 – 65	
	Alternatively: Activate "Start automatically" option – device starts measurement automatically after filling and calibration		86ff	64 – 65	

Step	Action	Chapter	Page	Figure	Checklist
10	For methanol | ethanol only: Connect enzyme reactor	6.2.5	52ff	34 – 36	
	Install enzyme reactor into tubing set		52ff	34 – 36	
11	Check measuring chamber	5.3 and 6.2.6.4	34 | 60	-	
	Check measuring chamber for absence of air bubbles | rinse if necessary		34 | 60	-	
12	For dialysis only: Perform reference measurement	7	88ff	66 – 69	
	Perform reference measurement with external sample or known measurement value		88ff	66 – 69	
13	Optional: Connect analog output to process control system	6.6	82	62	
	Connect analog output (0 – 10 V, 4 – 20 mA bzw. 0 – 20 mA)		82	62	

Table 7: Checklist for Setting-up Operation

9. Troubleshooting

If the BioPAT® Trace cannot be started up or malfunctions occur while running operations, then it is generally best to call upon experienced users for maintenance and | or the manufacturer's service technicians for repairs.

To avoid life-threatening hazards from an electric shock,

- **work on the electrical equipment should only be carried out by qualified and authorized experts!**
- **You should only try to remedy malfunctions that are obviously attributable to operating or servicing errors yourself!**
- **Under no circumstances should you try to troubleshoot any problems with electrical equipment!**

If you have problems with BioPAT® Trace, start by consulting the table below. It contains an overview of potential malfunctions:

Error | Error message	Possible Causes
Calibration failed	Calibration standard empty
	Transport buffer empty
	Air bubble inside the measuring chamber
	Incorrect tubing set used
	Measurement cell not properly inserted
	Wrong method selected
	Valve tubes inserted incorrectly or not inserted
	Dialysis only: Three-way stopcock incorrectly adjusted
	Tubing set out of date
	Leaks in the tubing set
	Calibration line blocked or tubing set clogged
	Software versions of device | PC not compatible
	Valve (yellow) cannot be triggered
Measured value too high or too low	Calibration erroneous
	"Standard" parameter entry and actual standard do not match
	Air bubble inside the measuring chamber
	Transport buffer empty
	Leaks in the tubing set
	Dialysis only: Reference measurement not performed
	Dialysis only: Reference measurement not up-to-date
No or negative measured value	Measurement cell not properly inserted
	Transport buffer empty
	Tubing set clogged
	Leaks in the tubing set
	Tubing set out of date
	No sampling probe or wrong sampling probe connected

Error | Error message	Possible Causes
Measured value varies greatly	Air bubble inside the measuring chamber
	Measurement cell not properly inserted
	Tubing set out of date
	Filtration sampling probe sealed and | or green valve of valve tube not properly inserted
No connection to Trace_mon	IP address set incorrectly
	PC: IP address not automatically assigned
	Incorrect or defective power cord
	Device boots but program does not start
Pump is not turning	Remove pump cassette and clean motor shaft
	Pull tight pump tube
No flow	Transport buffer empty
	Calibration standard empty
	Filtration probe blocked
	Filter clogged
	Tubing set clogged
	Leaks in the tubing set

Table 8: Problem description and troubleshooting on the BioPAT® Trace

Error message: Calibration failed

	Possible Causes	What should you do?
1.	The transport buffer is empty (Is there still enough transport buffer in the storage vessel? Is the suction tubing still in the storage vessel? Is buffer dripping into the drip counters?).	Connect new transport buffer and fill the tubing set.
2.	A calibration solution is empty.	Connect new calibration solution.
3.	Silicone tubes are not properly inserted into the valves.	Check if the valve tubes are secure and correct accordingly.
4.	There is an air bubble inside the measuring chamber.	Remove the air bubble through the rinsing line by using a syringe.
5.	Tubing set and adjusted application do not match (e.g. dialysis tubing set and filtration adjusted at the device).	Adjust application properly.
6.	A tube is pinched (e.g. when screwing the covers in the standard bottles).	Remove pinch.
7.	There is clogging in the tubing set (When the buffer remains in the tubing set over an extended period of time, salts begin to crystallize out. Crystallization usually occurs on the silicone tubes in the valves. Clogging can also occur due to microbial growth.).	Remove clogging. If this is not possible, use a new tubing set.
8.	Leaks (If the clog is not removed, tubes may burst.).	Correct leaks. If this is not possible, use a new tubing set.
9.	Sensor does not have proper contact (When the sensor is not inserted properly, the reverse side of the sensor does not have proper electrical contact with the sensor holder.)	Remove sensor and reinstall it properly.

	Possible Causes	What should you do?
10.	The dialysis membrane was installed in the probe in the incorrect position (only for DIALYSIS application).	Correctly install a new dialysis membrane into the probe.
11.	Three-way stopcock was not properly adjusted (only for DIALYSIS application. An external sample was measured and the three-way stopcock was not reset).	Adjust the three-way stopcock to the correct position.
12.	The software versions of the BioPAT® Trace and the PC are not compatible.	Use suitable software. If necessary, reinstall the PC software "trace_mon" from the website.

Table 9: Identifying and correcting calibration faults

10. Care and Maintenance

10.1 Device

If a measurement series is interrupted for a short time (maximum of two days), the system can continue to be operated without replacing the tubing set.
If the BioPAT® Trace is to be decommissioned for longer periods of time (longer than 7 days), the tubing set should be replaced upon recommissioning.

The surface of the BioPAT® Trace housing should only ever be cleaned with a wet cloth.

10.2 Tubing Set

Clogged or damaged tubing sets should be replaced with new ones.

Before every startup of the system, check all connections made with Luer lock or UNF fittings for any leaks and tighten them as necessary.

11. Appendix

11.1 Data Sheet

Measuring principle	Enzymatic-amperometric
Measuring range (glucose)	0.10 – 40 g/L
Measuring range (lactate)	0.05 – 5 g/L
Measuring range (methanol)	0.5 – 20 g/L
Measuring range (ethanol)	1 – 40 g/L
Measurement frequency	Up to 60 measurement values/h
Measurement deviation (glucose)	≤ 3% at 5.0 g/L
Measurement deviation (lactate)	≤ 3% at 2.5 g/L
pH range of the medium	4.8 – 9.2
Service life of the enzyme electrode	30 days or 5000 analyses
Ambient temperature	15 – 35 °C
Ambient humidity	10 – 90%
Interfaces	RS232, Ethernet
PC for control	Operating system Windows XP, Windows 7
Analog output	0..10 V | 0..20 mA | 4..20 mA
Input voltage	100..120 | 220..240 V ~; 50/60 Hz
Dimensions in mm (W × H × D)	120 × 170 × 200
Weight	1.8 kg

Table 10: Technical Data Sheet

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